NUTRITIONAL STRATEGIES OF ECHINOPLUTEI

By

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NUTRITIONAL STRATEGIES
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Maternal provisioning of the egg determines offspring characteristics which affect developmental success. Traditional life history models predicted that the extremes of larval strategies, planktotrophy (with small eggs) and nonfeeding lecithotrophy (with large eggs) would be favored by selection. This study discovered a range of egg sizes and nutritional strategies among species with planktotrophic larvae.

Nutritional experiments on echinoids (egg diameters from 76µm to 284µm) were done to examine the relationships among endogenous reserves, the need for exogenous nutrition, and developmental success. Different species and concentrations of algae were fed to echinoplutei. Differences were found among algal species in their suitability as food and in the concentrations required to support development in a limiting vs.

nonlimiting manner. Differences in timing of larval stages were noted among siblings fed different diets.

Eight species of echinoids allowed interspecific comparisons of the effect of endogenous reserves on development. Egg size determined the developmental stage reached without feeding and the rate of development with or without feeding. Larger egg sizes were correlated with relatively longer facultative feeding periods, and with shorter development times, but did not result in larger juveniles.

Blastomere isolations allowed intraspecific comparisons of the effect of differences in endogenous reserves on development. Endogenous reserves determined the developmental stage that could be reached without feeding. Starved larvae from half-size eggs of *Mellita quinquesperforata* (88μm) could only attain the 4-arm stage, while those from full-size eggs (110μm) reached a later stage (6-arm) on endogenous reserves. Larvae from half-size eggs grew longer feeding structures than did full-size siblings. Larvae from a species with larger eggs (*Encope aberrans*), from half (150μm) or full (195μm) eggs reached the final larval stage (8-arm) without feeding, and there was no evidence of differences in arm growth between fed treatments. There was no difference in juvenile size between egg size treatments within either species, when metamorphosis occurred at the onset of competency.

This work provides the basis for new directions in life history theory. Recent advances in life history modeling now recognize the advantages conferred by intermediate levels of maternal investment which allow a period of facultative feeding

CHAPTER 1
INTRODUCTION

A central assumption of life history theory is that maternal investment determines offspring fitness (Vance, 1973a, b). In free spawning marine invertebrates, the egg contents make up the entire maternal investment. Traditional life history models predicted that only the extremes of egg size would be favored by selection (Vance, 1973a, b; Christiansen & Fenchel, 1979, for a review see Havenhand, 1995). The bimodal distribution of egg sizes in some marine invertebrates (e.g. in echinoid echinoderms, Emlet *et al*., 1987) has been interpreted as empirical evidence supporting these life history models.

Not surprisingly, a commonly recognized pattern in the ecology of marine invertebrate larvae is that of two contrasting types of pelagic larval development: feeding (planktotrophic) larvae (from small eggs) and nonfeeding (lecithotrophic) larvae (from large eggs) (Thorson, 1950, Mileikovsky, 1971, Grahame & Branch, 1985, Levin & Bridges, 1995). Because of the predictions of life history models, any intermediate type of development was expected to be rare. However, an intermediate type, the facultative planktotroph, has been found in gastropod molluscs (Thompson, 1958, Kempf & Todd, 1989, Kempf & Hadfield, 1985, Perron, 1981, Kohn & Perron, 1994) and echinoid echinoderms (Strathmann, 1978a, Emlet, 1986, Hart, 1996). This type (facultative planktotroph) has been recognized as a functional lecithotroph because it can reach

metamorphosis without feeding, but it is a feeding larva (Herrera *et al.*, 1996, McEdward & James, 1997, McEdward, 1997).

Recently, a number of species of obligately planktotrophic subtropical echinoids with different degrees of dependence on feeding have been discovered (Eckert, 1995; McWeeney, 1995, Herrera *et al*, 1996). These larvae develop beyond the initial larval feeding stage on maternal reserves (without feeding), and have the capacity to feed facultatively before they reach the point of needing exogenous sources of nutrition (Herrera *et al.*, 1996). McEdward's life history model (1997) examines the possible advantages of a period of facultative feeding during larval development, and predicts that intermediate egg sizes can confer maximum reproductive success. My studies on several species of echinoids from the subtropical Gulf of Mexico with a range of egg sizes provide the basis for this latest advance in life history modeling.

Both endogenous and exogenous sources of nutrition affect the growth and development of planktotrophic larvae (Boidron-Metairon, 1988, Smirvo & McEdward, 1988; Strathmann *et al*, 1992, Fenaux *et al*, 1994, Eckert, 1995, Herrera *et al*, 1996). To examine the effects of differences in endogenous and exogenous nutrition amounts and/or sources on larval development, a series of comparative studies using several species of sea urchins was done. Echinoid larvae are particularly suitable for studies of nutritional strategies. They can be reared quickly and easily on any of a variety of algal species, and they have indeterminate development, which allows the experimental manipulation of egg size via blastomere isolation.

The effects of differences in exogenous nutrition were examined by manipulating the species and concentration of algae provided as larval food. The effects of differences

in endogenous reserves were evaluated among species with different egg sizes and within species by experimental manipulation of egg size. These studies were undertaken to address the following questions: What is an insufficient versus limiting versus non-limiting diet for echinoplutei, and what are the effects of those diets on larval development and metamorphosis? How do non-limiting concentrations of different food species affect larval development time and trajectory? How does maternal investment affect life history traits? Can larvae from species with larger egg sizes reach later stages of development without depending on exogenous feeding, and are they less affected by differences in exogenous sources during any particular stage of their development? Can a non-limiting source of exogenous food compensate for lower levels of egg energy content? How does a change in maternal investment affect the degree of dependence on exogenous food? Is the increase in maternal investment seen in species with intermediate degrees of planktotrophic development limited to what is required for intrinsic maximal development?

This dissertation is made up of seven chapters. There is an introductory chapter, five chapters of experimental studies, and a summary chapter. Each of the second through sixth chapters describes a separate study on the nutritional strategies employed by echinopluteus larvae. This introduction serves mainly an organizational purpose.

The experiments in Chapter 2 allow comparisons of how exogenous nutrition affects total larval development time, the timing of larval stages, and the size of the juvenile at metamorphosis. These experiments also reveal whether non-limiting amounts of exogenous food can compensate for a relatively small egg size. That chapter is a comparative study of the effects of different species and concentrations of algae provided

as food to echinopluteus larvae of the sea urchin *Lytechinus variegatus* (Lamarck). Three species of algae, *Rhodomonas lens* (Pasher and Ruther), *Dunaliella tertiolecta* (Butcher), and *Isochrysis galbana* (Parke) were provided separately as food and in several concentrations. A second trial was conducted with 11 different concentrations of *D tertiolecta* presented as particulate food to the larvae. An evaluation of the concentration of each algal species necessary to provide an insufficient, limiting, or non-limiting diet is presented. *Lytechinus variegatus* was chosen because its larva is known to be an extreme planktotroph. For the purpose of the subsequent studies, the concentration and species of algae needed to provide an unlimited diet to an extreme planktotroph is assumed to be sufficient to provide an unlimited diet to any planktotrophic echinoid larva.

The results in Chapter 3 reveal the effects of a non-limiting diet versus starvation, how non-limiting exogenous energy sources affect larval development time and trajectory, and whether high levels of food can compensate for small egg sizes. This chapter is an extensive morphometric analysis of the bodies and skeletons of larvae of the sea urchin *Lytechinus variegatus* fed three different diets. Detailed comparisons of the growth and form of *L. variegatus* larvae with larvae of other species are also provided. The chapter also includes morphometric comparisons between larvae fed equal concentrations of two species of algae (*Rhodomonas lens* or *Dunaliella tertiolecta*), and between larvae which were starved and those which were fed non-limiting amounts of *D tertiolecta*. This chapter is in press in the Journal of Experimental Marine Biology and Ecology.

Chapter 4 examines the relationship between egg size and dependence on exogenous food in the larvae of eight species of subtropical echinoids. These results

illuminate how maternal investment affects life history traits, whether larvae from larger eggs can reach later stages without feeding, and what effect starvation has on larvae among species with different egg sizes. Larvae were either fed or starved. Stage of development attained, timing of developmental stages, time of metamorphosis, and size at metamorphosis were noted for each species of urchin. Differences in dependence on exogenous food in relation to egg size (maternal investment per offspring) are discussed in light of recent advances in models of marine life histories. An earlier version of this chapter is published in Volume 19 of Oceanologica Acta (1996).

Chapter 5 presents a discussion of the effects of a non-limiting diet versus starvation in an intraspecific comparison of larvae from full-size and half-size eggs. It reveals whether larvae from larger eggs can reach later stages of development without feeding, whether they are less affected by differences in exogenous sources during different stages in their development, whether a non-limiting source of exogenous nutrition can compensate for differences in egg size. It also illustrates the effect of starvation on larvae from different egg sizes. This chapter examines the effects of an experimental reduction in egg size on the larvae of the sand dollar *Mellita quinquiesperforata* (Leske). Larvae were reared from half-size and full-size eggs and were fed limiting or non-limiting amounts of *Dunaliella tertiolecta*, or were starved. This experiment was done in collaboration with S.K. McWeeney and a discussion of the effects of limiting versus non-limiting diet was presented in McWeeney (1995). *Mellita quinquiesperforata* was chosen for this experiment because its larvae from full-size eggs can reach the 6-arm stage without feeding. This is a significant intermediate stage in larval development because there is an increase in metabolism between the 4-arm and 8-

arms stages (McEdward, 1985a) and an increase in the amount of nutritional resources required to support development at the 6-arm stage (Fenaux *et al* , 1988) Attaining the 6-arm stage requires a large investment in energy

The sand dollar studied in this chapter was classified as *M quinquiesperforata* according to the criteria of Serafy (1979) A more recent work by Harold and Telford (1990) distinguishes between the species *M. quinquiesperforata* and *Mellita tenuis* based on several morphometric parameters of the sand dollar test. However several morphometric measurements of the sand dollars used in these studies revealed intermediate values between those given for *M. quinquiesperforata* and *M tenuis* by Harold and Telford (1990) (unpublished data)

Chapter 6 addresses the questions: how does maternal investment affect life history traits, do larvae from larger egg sizes reach later stages without feeding, and are they are less affected by exogenous sources during any stages of development, can high exogenous food concentrations compensate for lower egg energy content, does a change in maternal investment affect the degree of dependence of exogenous food, and is the amount of energy packaged in the egg limited to that required for intrinsic maximal development? That chapter is a study of the effects of an experimental reduction in egg size on the larvae of the sand dollar *Encope aberrans* Martens Larvae were produced from full-size and half-size eggs, and were either fed non-limiting amounts of *Dunaliella tertiolecta*, or were starved A detailed morphometric analysis of the growth and form of larvae of *Encope aberrans* from each treatment is presented That chapter provides an intraspecific comparison of the effects of a non-limiting diet or starvation on larvae from full-size and half-size eggs in a species with a long facultative feeding period and an egg

size well above the minimum required for the development of the initial feeding larval form.

In many of the experiments in Chapters 2-6, each experiment was conducted on larvae from a single spawn and a single pair of parents. This reduces the amount of variation due to genotype because all of the larvae within each of these studies are full siblings. Although egg size is correlated with egg energy content among many species (Strathmann & Vedder, 1977, Turner & Lawrence, 1979) it has been shown that there is considerable variation in maternal investment among the eggs of single spawns in some echinoderms (McEdward & Coulter, 1987, McEdward & Carson, 1987). The variation in energy content among the eggs of a single spawn from a single urchin is as great as the variation in maternal investment among the eggs of multiple females in a population and among the eggs of females from different populations (McEdward & Carson, 1987). That the variation in egg energy content within a single spawn reflects the variation within the species is assumed to be the case for those species studied in this dissertation.

While egg energy content is not correlated with egg size within species, both of these characteristics are continuous variables and are normally distributed (McEdward & Carson, 1987). Embryos from blastomere isolation treatments will have (on average) half of the energy content of embryos from full-size eggs, and differences between the larvae from each treatment can be assumed to be the result of a reduction in maternal investment.

Blastomere isolations allow us to directly observe and measure the effects of a change in maternal investment within a species. These intraspecific comparisons can be used to predict the effects of an evolutionary change in egg energy content. Previously,

these predictions relied on inference from interspecific comparisons Interspecific comparisons are likely to be confounded by differences due to genetics, evolutionary history, taxonomy, geography, and seasonality Intraspecific comparisons on full siblings reared under identical conditions allow us to isolate changes in development that are due strictly to a decrease in maternal provisioning of the egg

Chapter 7 is a summary discussion of the major conclusions from each chapter Dependence on exogenous versus endogenous nutrition is discussed in relation to marine invertebrate life histories.

CHAPTER 2
DEVELOPMENT AND METAMORPHOSIS OF *LYTECHINUS VARIEGATUS* IN RESPONSE TO VARIATION IN EXOGENOUS NUTRITION

Introduction

The developmental patterns of planktonic larvae of benthic marine invertebrates are categorized depending on the nutritional sources utilized for development to metamorphosis (Thorson, 1950; Mileikovsky, 1971, 1974, Chia, 1974, Jablonski & Lutz, 1983, Grahame & Branch, 1985, Levin & Bridges, 1995) Planktotrophs require exogenous particulate food to support larval development to metamorphosis. Lecithotrophs (both feeding and nonfeeding) can develop to metamorphosis utilizing egg energy contents (maternal reserves), and the formation of the juvenile does not require external particulate nutrition.

The growth and development of the planktotrophic larvae of echinoid echinoderms (echinoplutei) have been extensively studied for at least a century (Bury, 1895, Mortensen, 1898) The ease with which gametes and larvae can be manipulated and reared in the laboratory has made this group a convenient model for studies in development, larval ecology, and marine invertebrate life history evolution.

Species with planktotrophic larvae have less maternal reserves in the egg than do those with lecithotrophic larvae, and begin exogenous feeding when larval feeding structures develop. Within the echinoids, feeding begins when one or two pairs

of larval arms appear (2- or 4-armed pluteus stage [2pl or 4pl]) (Okazaki, 1975; Strathmann, R.R.,1987) In many species with small eggs, development beyond this stage requires the ingestion of exogenous particulate food, otherwise planktotrophic echinoid larvae deteriorate and die (Fenaux *et al.*, 1988), as do the planktotrophic larvae of other taxa, e.g., crustaceans (Anger & Spindler, 1987) and molluscs (His & Seaman, 1992)

Planktotrophic larvae exhibit significant developmental responses to a number of environmental conditions including temperature (McEdward, 1985a) and the concentration of exogenous particulate food. A limited food supply alters the morphology and development time of echinoid larvae Arm length, ciliated band (feeding structure) length, and time to metamorphic competence increase when larvae are reared in nutritionally limiting conditions (Paulay *et al* , 1985, Boidron-Metairon, 1988) These morphological and developmental responses to exogenous food concentration (phenotypic plasticity) are the focus of many recent studies (Strathmann *et al* 1992, Fenaux *et al* , 1994, Eckert, 1995, McWeeney, 1995, Herrera, in preparation).

Comparisons are often made within and among species and studies. Theories of larval ecology and life history evolution are based on these comparisons. Larvae in various studies are reared on different amounts and different species of algae Comparisons among studies and reviews of echinoid development do not account for the changes in development and the time to metamorphic competence caused by differences in diet (Emlet *et al* , 1987, Strathmann, R.R., 1987, Pearse & Cameron, 1991)

The effect of temperature on development is widely recognized, and the fact that nutrition also has some effect is often mentioned, yet no apparent effort has been made to

define a limited vs. unlimited vs. inadequate diet for each species. In fact, requirements for exogenous food may not be the same, even among those species with similar egg sizes, due to differences in temperature, seasonality, or phylogeny. For more valid comparisons of larval development, the effects of various diets need to be defined. What are the minimum concentrations of various algal species necessary to support development through metamorphosis? What are the minimum concentrations to support maximal rates of development to metamorphosis? Are there differences among the algal species in their quality as larval food? Does the concentration of algae fed to larvae have an effect on juvenile size? How do these findings compare to those of previous studies?

In this study, larvae of the sea urchin *Lytechinus variegatus* were raised under several nutritional conditions, varying the species and concentration of algal cells provided as food. An inadequate diet is any concentration of food at which the larvae fail to reach metamorphic competence. A nutritionally limiting diet is defined as any concentration of food that is below the amount required for the most rapid development of *L. variegatus* larvae through the characteristic larval stages and to metamorphic competence. An unlimited diet is defined as any concentration of food at which (or above which) the larvae develop and metamorphose at maximum rates.

Methods

Adults of the sea urchin *Lytechinus variegatus* (Lamarck) were collected from subtidal populations by dredge at depths of 2 to 3 meters off Seahorse Key, Florida (29°06'N, 83°03'W), in May, 1992 and by SCUBA approximately 8 miles offshore from Hudson, Florida, in the Gulf of Mexico (28°22.92"N, 82°52'00"W), in May, 1993. The

animals were transported to the laboratory at the University of Florida, Gainesville, and maintained in a recirculating seawater system (average temperature 22°C, average salinity 34ppt.). Seawater for larval and algal culturing was collected at the University of Florida Whitney Marine Laboratory, Marineland, Florida (Atlantic coast) Spawning, fertilization, and larval culturing utilized methods by M.F Strathmann (1987) Adults were induced to spawn by injection of 0.5ml of 0 55M KCl into the coelomic cavity Females were inverted over beakers of filtered seawater (0 45 µm) to collect the eggs. Eggs were then rinsed three times in clean filtered seawater Egg diameters were measured with an ocular micrometer on a compound microscope. Males were inverted over petri dishes and the sperm collected "dry" Two drops of concentrated sperm were diluted in 10ml of filtered seawater just prior to fertilization. A few drops of the dilute sperm suspension were added to the cleaned eggs in filtered seawater in a 250ml beaker. Eggs were observed just after fertilization to evaluate fertilization success. A visible vitelline envelope rises from the surface of the eggs that have been fertilized In all experiments, fertilization success was greater than 95%

Approximately 1000 embryos were raised to the early two-armed pluteus stage (2pl) in filtered seawater in each of several glass culture vessels (250ml) At the 2pl stage larvae were placed in culture vessels at concentrations of 20 per 200 ml filtered seawater (0 45µm) and nutritional treatments were initiated. The culture containers were placed in an environmental chamber and maintained with constant illumination. Cultures of embryos and larvae were unstirred The culture water was changed every second day and freshly washed algal cells were added at each water change. For each water change, larvae were concentrated by reverse filtration (Boidron-Metairon, 1988), transferred to a

small dish, and observed under the stereoscope. Freshly filtered seawater and washed algal cells were placed in clean containers and larvae were individually pipetted into the new culture containers and counted to measure survival.

Algae were raised according to the methods of Guillard (1975). In preparation for larval feeding, algal cells were washed by centrifuging, decanting away the algal nutrient medium, resuspending the algal cells in filtered seawater, recentrifuging the suspensions, removing the washwater and then resuspending the algal cells a second time in freshly filtered seawater. Algal concentrations were determined following procedures outlined in Guillard (1981) using a haemacytometer with improved Neubauer ruling.

The effect of diet (algal species and concentration) on larval survival and development rates was examined in two separate trials. The first trial (May, 1992) tested the effect of varying algal species and cell concentration in the cultures. These larvae were from the single spawn of one pair of parents. Larvae were fed monocultures of *Rhodomonas lens* (Pasher and Ruther), *Dunaliella tertiolecta* (Butcher), or *Isochrysis galbana* (Parke). These species were selectrd because they are commonly used to culture marine invertebrate larvae. *Rhodomonas lens* has been shown to support development through metamorphosis in *L. variegatus* at rates similar to those in the environment at some times of the year (Boidron-Metairon, 1987). Each algal species was fed to separate cultures of larvae at concentrations of 2, 4, 6, and 8 cells μl^{-1}. Control cultures were starved. Each treatment was duplicated. Larvae were raised at a temperature of 25°C. Larval survival and developmental stage were recorded twice daily at twelve-hour intervals. After rudiment formation was observed, larvae in one of each duplicate treatment were periodically tested for metamorphic competency. The larvae were

exposed to excess KCl (40mM, 15 minutes), and then observed for metamorphic activity for two hours and again after twelve hours

The second trial (May 1993) tested the effect of smaller variations in algal cell concentrations of a single species (*Dunaliella tertiolecta*) This alga was fed to cultures of larvae in concentrations of 0, 0 5, 1, 2, 3, 4, 6, 8, 10, 12, and 14 cells μl^{-1} These larvae were from the single spawn of one pair of parents. These parents were not the same pair of urchins that was used in the first trial. Larvae were raised at a temperature of 27°C. Other culturing and observational procedures were the same as in the first trial. Larvae were observed twice daily, and survival, larval stage, time of development of the juvenile rudiment, time of metamorphosis, and size at metamorphosis (diameter of the test without spines) were recorded Some larvae were maintained in culture for several days; metamorphosis was induced and juvenile size measured at later ages (12 or 17days)

Statistical analyses included descriptive statistics, one and two factor ANOVA's, and Duncan's New Multiple Range Tests. ANOVA's were performed using SuperANOVA (Abacus Concepts, Inc., Berkeley, CA, 1989) A significance level of $p \leq 0.05$ was used for all analyses. Juvenile test diameters are reported in micrometers with standard error values.

Results

All larvae were at the 2pl stage at 24 hours and all were at the 4-armed (4pl) stage at 2, 3, and 3 5 days. Starved larvae reached only the 4pl stage. Larval development was synchronous within each treatment. There was no variation in stage within the treatments

In larvae fed *D. tertiolecta* (Table 1), the larvae fed 2 cells μl^{-1} reached the 8-armed (8pl) stage at 7.5 days. However, they did not show any visible evidence of juvenile rudiment formation. The larvae fed 4 cells μl^{-1} reached the 8pl stage at 7 days and metamorphosed at 14.5 days. Larvae fed 6 cells μl^{-1} were 8pl by day 6.5 and metamorphosed at 14.5 days. Larvae fed 8 cells μl^{-1} were 8pl at 6 days and metamorphosed at 13.5 days.

Larvae fed 2 cells μl^{-1} of *R. lens* (Table 1), reached the 8pl stage at 11.5 days, but did not show any visible evidence of juvenile rudiment formation. The larvae fed 4 cells μl^{-1} reached the 8pl stage at 7.5 days and metamorphosed at 14.5 days. Larvae fed 6 cells μl^{-1} were 8pl by day 6 and metamorphosed at 14.5 days. Larvae fed 8 cells μl^{-1} were 8pl at 6 days, and metamorphosed at 13.5 days.

Larvae fed 2 cells μl^{-1} *I. galbana* (Table 1) did not reach the 6pl stage and did not show any visible evidence of juvenile rudiment formation. The larvae fed 4, 6 or 8 cells μl^{-1} reached the 6pl stage at 8 days, the 8pl stage at 12 days, but did not develop a visible juvenile rudiment, and did not metamorphose.

In the second trial larvae were fed 11 different concentrations of *Dunaliella tertiolecta* (Table 2). The diets ranged from no food to 14 cells μl^{-1}. All larvae were at the 2pl stage by 24 hours, and all were 4pl by day 2. Starved larvae reached only the 4pl stage. Larvae fed 0.5 cell μl^{-1} reached the 8pl stage at 8 days, but did not develop a juvenile rudiment. Larvae fed 1 or 2 cells μl^{-1} took 5 days to reach the 8pl stage. Larvae fed 1 or 2 cell μl^{-1} showed some rudiment formation, but none of the larvae on these two diets metamorphosed. Larvae fed 3 cells μl^{-1} reached the 8pl stage at 5 days and metamorphosed at 17 days. Larvae fed 4 or more cells μl^{-1} were 8pl at day 4.5. Those

Table 1 Time to characteristic larval stages and metamorphosis in larvae of *Lytechinus variegatus* reared at 25°C, fed 5 concentrations of each of the unicellular algal species*Dunaliella tertiolecta*, *Rhodomonas lens* and *Isochrysis galbana* (Key to larval stages· 6pl = 6-armed pluteus, 8pl = 8-armed pluteus, 8R = 8-armed pluteus with visible signs of juvenile rudiment) For each larval stage n=40 Metamorphosis as reported is the day on which 50% or more of the larvae metamorphosed

days to larval stages and metamorphosis

diet	6pl stage	8pl stage	8R stage	metamorphosis
D tertiolecta (cells ul^{-1})				
0	-	-	-	-
2	5	7 5	-	-
4	4 5	7	13	14 5
6	4 5	6 5	9 5	14 5
8	4	6	9	13 5
R lens (cells ul^{-1})				
0	-	-	-	-
2	6	11 5	-	-
4	5 5	7 5	10	14 5
6	4	6	8	14 5
8	4	6	7 5	13 5
I galbana (cells ul^{-1})				
0	-	-	-	-
2	-	-	-	-
4	8	12	-	-
6	8	12	-	-
8	8	12	-	-

Table 2. Time to characteristic larval stages and metamorphosis, and size at metamorphosis in larvae of *Lytechinus variegatus* reared at 27°C, fed 11 concentrations of *Dunaliella tertiolecta* n = 40, unless otherwise specified Metamorphosis as reported is the day on which at least 50% of the induced larvae metamorphosed, n = 10 For juvenile size n = 6-9, except n = 4 for the 3 cells µl^{-1} treatment on day 17.

D. tertiolecta	Number of days to stage				Juvenile size (µm) at day		
cells µl^{-1}	6pl	8pl	8R	Metamorphosis	11	12	17
0	-	-	-	-	-	-	-
0.5	7	8	-	-	-	-	-
1	4.5	5	11.5	-	-	-	-
2	4.5	5	8.5	-	-	-	-
3	4.5	5	7.5	17 (40%)	-	-	324±18
4	4	4.5	7.5	12	-	403±3	431±1
6	4	4.5	7.5	12		399±1	455±6
8	4	4.5	6.5	11	398±2	451±5	-
10	4	4.5	6.5	11	396±2	437±6	-
12	4	4.5	6.5	11	397±1	437±6	-
14	4	4.5	6.5	11	396±1	439±11	-

fed 4 or 6 cells μl^{-1} metamorphosed at 12 days. Those larvae fed 8 to 14 cells μl^{-1} metamorphosed at 11 days

Juveniles from larvae fed 3 cells μl^{-1} had test diameters of 324±18 µm (day 17). Juveniles from larvae fed 4 cells μl^{-1} had test diameters of 403±3 µm when metamorphosis occurred on day 12 and diameters of 431±1 µm when metamorphosis occurred on day 17. Juveniles from larvae fed 6 cells μl^{-1} had test diameters of 399±1 µm when metamorphosis occurred on day 12 and diameters of 455±6 µm when metamorphosis was induced on day 17. Juveniles from larvae fed 8 to 14 cells μl^{-1} had test diameters of 396-398 ±1-2 µm when metamorphosis occurred on day 11. When metamorphosis was induced on day 12 juveniles from larvae fed 8 cells μl^{-1} had test diameters of 451±5 µm, those raised on 10 cells μl^{-1} had test diameters of 437±6 µm, juveniles from larvae fed 12 cells μl^{-1} had test diameters of 437±6 µm, and those from larvae fed 14 cells μl^{-1} had test diameters of 439±11 µm.

Discussion

Isochrysis galbana is an insufficient diet for the larvae of *Lytechinus variegatus*. Concentrations of 2 cells μl^{-1} and below were insufficient to support larval growth and development beyond the 4-armed stage, a stage that the larva can attain without feeding. While concentrations of 4 to 8 cells μl^{-1} will support development of the larval body to the 8-armed stage, the larvae retain a simple early 8-armed larval shape and fail to develop the subsequent elaborations of the larval body seen in well nourished individuals

(epaulettes, pedicellariae). In addition, there is no evidence of juvenile rudiment formation.

Rhodomonas lens and *Dunaliella tertiolecta* at a level of 4 cells μl^{-1} and above are sufficient diets for larval development through metamorphosis. However, diets of *R. lens* at concentrations below 6 cells μl^{-1} and diets of *D. tertiolecta* at concentrations below 8 cells μl^{-1} are limiting to the development of the larval body. Larvae fed diets below these levels require more time to achieve each of the later larval stages than do those larvae fed higher concentrations of the same algal species. Diets below 8 cells μl^{-1} of each of these species are also limiting to rudiment formation. Those larvae fed less than 8 cells μl^{-1} do not begin to form the juvenile rudiment as early as do larvae fed 8 cells μl^{-1} or more.

Eight cells μl^{-1} of *Dunaliella tertiolecta* appears to be comparable to a diet of 6-8 cells μl^{-1} of *Rhodomonas lens* for development to the fully formed 8-armed pluteus. However, a sufficient diet of *R. lens* (6-8 cells μl^{-1}) appears to allow the initiation of the juvenile rudiment at an earlier age than does a diet of *D. tertiolecta*. Metamorphosis occurred at the same age in both of these treatments. At lower concentrations (2 and 4 cells μl^{-1}), larvae fed *D. tertiolecta* reached the 6pl and 8pl stages sooner than do those fed the same concentrations of *R. lens*. These larvae fed *D. tertiolecta* exhibit an acceleration of larval arm development as an expression of developmental flexibility (McEdward & Hadfield, 1996). This flexibility may have effects similar to those of larval arm elongation (morphological plasticity) seen in other studies of larval responses to variations in diet (Boidron-Metairon, 1988; Strathmann *et al.*, 1992; Fenaux *et al.*,

1994). The early addition of larval arm pairs increases the length of the ciliated band and allows larvae to capture more food.

The first trial revealed that *Isochrysis galbana* is an insufficient diet at any concentration studied. *Rhodomonas lens* provides an unlimited diet at concentrations of 6 cells μl^{-1}, a limited diet at concentrations of 4 cells μl^{-1}, and an insufficient diet at concentrations of 2 cells μl^{-1}. Larvae on a sufficient diet of this alga reached metamorphosis at 13.5 - 14.5 days. This time to metamorphosis on a diet of *R. lens* is similar to that obtained in Boidron-Metairon's (1987) study (13 days), and also to development times obtained for larvae fed natural seawater in that same study (16 days). *Dunaliella tertiolecta* is an unlimited diet at concentrations of 8 cells μl^{-1}, a limited diet at 4 cells μl^{-1}, and an insufficient diet at 2 cells μl^{-1}. This alga had previously been found to be an insufficient diet, even at "excess" concentrations (Boidron-Metairon, 1987), and at 10 cells μl^{-1} (McEdward & Lawrence, 1981). The concentrations used in Boidron-Metairon's study (1987) were not reported and may have been lower than "excess" for this species. Extremely high concentrations of larvae affected results in the McEdward and Lawrence study (1981).

In the second trial, 8 cells μl^{-1} of *Dunaliella tertiolecta* was confirmed as an unlimited diet for the development of both larval and juvenile structures. Diets of 4 cells μl^{-1} and above were unlimited diets for the growth and development of a fully-formed larva. However, less than 8 cells μl^{-1} is a limiting diet for the rapid growth of the juvenile. Two cells μl^{-1}, or less, is insufficient for full development to the juvenile (Table 2).

Although 3 cells μl^{-1} supported development to metamorphosis in a few of the larvae in that treatment, these larvae were not competent to metamorphose until day 17, 6-7 days longer than required when diets were 4-14 cells μl^{-1}. The juveniles from the 3 cells μl^{-1} treatment were significantly smaller than those in any of the other treatments (regardless of the day of metamorphosis in those treatments).

Larvae fed 4-6 cells μl^{-1} reached competency one day later (12d) than those fed 8-14 cells μl^{-1}. They also metamorphosed into juveniles of the same size as those fed diets of 8-14 cells μl^{-1}, which were induced to metamorphose on day 11, but smaller than those fed 8-14 cell μl^{-1}, in which metamorphosis was induced on day 12. Larvae fed 8-14 cells μl^{-1} were competent to metamorphose by day 11. Larvae from those treatments induced to metamorphose on day 12 were larger than those from day 11. A longer feeding period in any treatment resulted in a larger juvenile at metamorphosis. Given sufficient food, delaying metamorphosis allows the larvae to attain a larger juvenile size upon metamorphosing. Eight cells is a nonlimiting diet, and higher concentrations of food did not allow the larvae to attain metamorphosis earlier nor to metamorphose into a larger juvenile.

With the exception of individuals fed the 3 cells μl^{-1} diet, juveniles from all treatments (4-14 cells μl^{-1}) were the same diameter when metamorphosis occurred on the first day of competency for each treatment. There is a relatively conservative juvenile size in this echinoid species at the onset of competency.

In the first trial, diets of 2 cells μl^{-1} of either *D. tertiolecta* or *R. lens* were insufficient for any visible development of the juvenile rudiment. The first trial was terminated at day 15. In the second trial, diets of 1 or 2 cells μl^{-1} of *D. tertiolecta* did

support some level of rudiment formation in some, but not all, of the larvae However, none of these larvae reached metamorphosis within the 30 days of the trial. The fact that larvae fed 1 or 2 cells μl^{-1} of *D. tertiolecta* in one trial showed some rudiment formation, while those fed 2 cells μl^{-1} in the other trial did not, may be due to differences in temperature or to differences in the endogenous reserves in the egg due to maternal nutritional condition (Thompson, 1983)

Comparisons among species are often made more difficult because of the differences in culturing conditions utilized by individual researchers (Emlet *et al.*, 1987, Pearse & Cameron, 1991). Temperature and diet have been demonstrated to have profound effects on the growth and development of echinoid larvae (McEdward, 1985a, Boidron-Metairon, 1988, Strathmann *et al.*, 1992, Fenaux *et al.*, 1994). Even within the species *Lytechinus variegatus*, separate studies have reported vastly different schedules of larval development to metamorphosis. *Lytechinus variegatus* larvae in the current study were raised at higher temperatures than in some previous studies (Boidron-Metairon, 1987, Mazur & Miller, 1971). However, in Boidron-Metairon's study (1987) temperatures were only 2-4°C lower and yet it was reported that *L. variegatus* did not develop a juvenile rudiment on "excess" (not defined) amounts of *Dunaliella tertiolecta*. In the present study at 27°C 3 cells μl^{-1} of *D. tertiolecta* is sufficient for development through metamorphosis within 17 days, and 8 cells μl^{-1} will support metamorphosis within 11-12 days. Mazur and Miller (1971) reared *L. variegatus* larvae to metamorphosis in 33-37 days at 23°C, however larvae in that study were fed an unspecified amount of a *Chlorella* like freshwater algae and it is unclear if water in the larval cultures was changed. *L. variegatus* larvae have been raised at 27°C on

Rhodomonas lens and reached metamorphosis within 9 days (Cameron *et al*., 1985, McEdward & Herrera, in press). There is a wide range of development times for *L. variegatus* among previous studies. Most of these studies did not report the concentration of algae provided as food for the larvae. Standardizing and reporting the levels of particulate food provided in larval cultures would facilitate comparisons of larval development among species and studies.

Defining diets is also important in studies on developmental plasticity. Identifying diets which are insufficient vs. limited vs. unlimited is essential to further understanding the developmental responses of larvae to their nutritional status. In this study, larvae fed *D. tertiolecta* progressed through the stages of the larval body more rapidly, yet they did not begin development of the juvenile rudiment as early, and they reached metamorphosis at approximately the same time as did those fed *R. lens*.

This study also supports the hypothesis that the building of the larval body is much less "energetically expensive" than building the juvenile rudiment (McEdward, 1984). Larvae of *L. variegatus* fed only 0.5 cells μl^{-1} of *D. tertiolecta* can reach the 8p1 stage, those fed 3 μl^{-1} cells can develop through metamorphosis, and those fed 8 cells μl^{-1} exhibit a maximal rate of development through metamorphosis.

The determination of the minimum concentrations of algal food species which are non-limiting to larval growth and development is important. Some algae may release metabolites which are poisonous to larvae (Wilson, 1981). Eckert (1995) reported that *I. galbana* may have been toxic to larvae *of Encope michelini* at concentrations of 50 cells μl^{-1}. Studies of growth under unlimited food conditions can be conducted and this problem with toxicity from high concentrations of algae can be minimized by feeding

larvae the minimum concentrations of algae which still allow unlimited growth and development

Defining insufficient vs. limited vs unlimited diets is essential for comparisons of larval growth and development between and among species and for a better understanding and definition of developmental plasticity Insight into various species requirements for exogenous particulate nutrition will contribute to our understanding of the diversity of energetic strategies among planktotrophic larvae and the energetic costs of larval vs juvenile development.

Chapter 3
BODY FORM AND SKELETAL GROWTH IN LARVAE OF THE SEA URCHIN *LYTECHINUS VARIEGATUS*

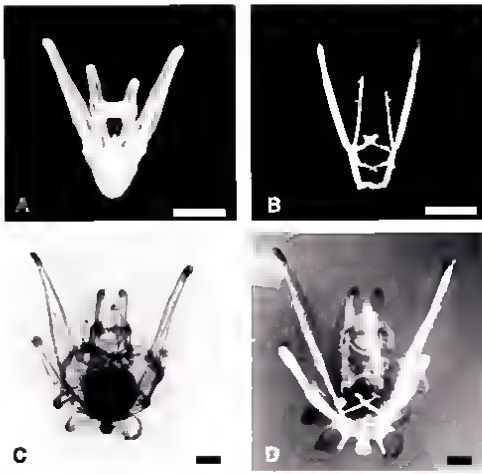
Introduction

There are many different planktotrophic (feeding) larval forms in the approximately 30 phyla of marine benthic invertebrates. Each form must solve the problem of feeding effectively on plankton while suspended in the water column. The planktotrophic pluteus larva of the echinoids has solved this problem with the use of a ciliated band feeding by capturing particles with a localized reversal of ciliary beat (Strathmann, 1971; Strathmann *et al.*, 1972). The total length of the ciliated band determines feeding ability (Hart, 1991)

The larva of the sea urchin *Lytechinus variegatus* develops four pairs of arms during the larval period (Fig. 1) The ciliated band runs around the larval body and up and down the larval arms (Fig 2) The arms of the *L. variegatus* larva are the postorals added during the 2pl stage, the anterolaterals added at the 4pl stage, followed by the posterodorsals at the 6pl stage and finally the preorals are added at the 8pl stage (Mazur & Miller, 1971) The larval body is continually growing and changing in shape

McEdward (1984) demonstrated that the feeding capability of echinopluteus larvae depends on the geometry of their growth. The length of the ciliated band increases with positive allometry in relation to body size. This is accomplished by the growth and

Figure 1 Pluteus larvae of the sea urchin *Lytechinus variegatus*. A. 4-armed stage, B 4-armed stage skeletal features, C 8-armed stage, D 8-armed stage skeletal features Scale bars = 100µm.


A
B
C
D

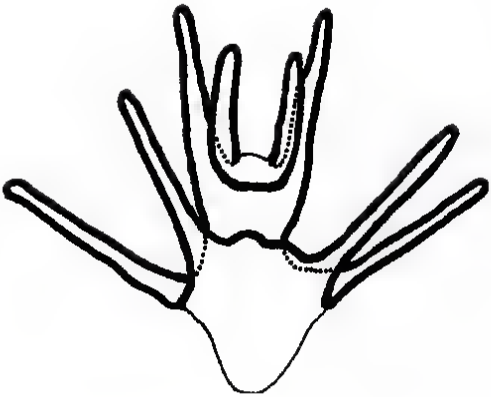


Figure 2 Location of the ciliated band (bold and dotted lines) on an 8-armed pluteus larva (ventral view), (modified from McEdward, 1984)

elongation of 1-6 pairs of larval arms, which greatly increases the length of the ciliated band feeding structure in proportion to larval body growth.

There are differences in the morphology of plutei fed different levels of food Larvae fed low levels of food, or starved, grow longer arms in proportion to their body length, and thus increase the amount of ciliated band feeding structure for their body size (Boidron-Metairon, 1988, Strathmann *et al*., 1992, Fenaux *et al*., 1994). When food levels are high, growth of the juvenile rudiment occurs earlier (Chapter 2) and larval structures are less developed (Strathmann *et al*., 1992) Growth and form of the larval body and development of the juvenile rudiment are not fixed processes affected only by genetics or endogenous reserves in the egg. Differences in exogenous food concentrations cause shifts in the timing of development and the extent of growth of the larval body

In this chapter I quantify the growth and form of the pluteus larva of the sea urchin, *Lytechinus variegatus* using three-dimensional morphometric measures of the larval body and skeleton. Measurements were done daily and over the span of development from the simple form of a 2pl larva with one pair of postoral arms to the complex form of an 8pl larva with four pairs of arms (Fig. 1). Most previous work has been done on cold temperate species of sea urchins (*Dendraster excentricus*, McEdward, 1984, 1985a, 1986a,b , Hart, 1991, *Strongylocentrotus droebachiensis*, McEdward, 1986a, b; Hart & Strathmann 1988, Sinervo & McEdward, 1988 *Strongylocentrotus purpuratus*, McEdward, 1986a, b, Sinervo & McEdward, 1988, *Strongylocentrotus franciscanus*, McEdward, 1986a, b). This study presents the first three-dimensional measurements of the growth and form of a subtropical echinoid This study is also the

first to detail larval skeletal morphometry in an echinoid. Larvae were cultured on two different species of unicellular algae or were starved providing a comparison of the effects of diet on size and shape changes during development, and evidence of morphological plasticity in response to starvation. This work also provides the basis for the first quantitative comparison of the growth and form of a subtropical echinoid species with cold temperate sea urchins.

Methods

Adults of the sea urchin *Lytechinus variegatus* (Lamarck) were collected using SCUBA in depths of 8-10 meters, 15-25 km offshore from Cedar Key, Florida (29°01.71'N, 83°12.03'W, July, 1992). Culture methods followed those outlined in Chapter 2.

At the 2pl stage larvae were placed in culture vessels at concentrations of 300 per 1.6 l filtered seawater and nutritional treatments were initiated. The culture containers were placed in an environmental chamber and maintained at 26°C with 24-hr illumination and without stirring. Water changes and feeding followed procedures outlined in Chapter 2. Larval mortality was <5%.

Larvae were raised in two separate trials. In the first trial, larvae were raised on 8 cells μl^{-1} *Rhodomonas lens* (Pasher and Ruther). In the second trial, in order to provide a comparison of the effect of food quality and to investigate the effects of starvation, full sibling larvae were either starved or were fed 8 cells μl^{-1} *Dunaliella tertiolecta* (Butcher). This concentration of these algal species was shown to be nonlimiting to larval development (Chapter 2). In both trials, larvae were raised under the same conditions.

Beginning with the 2pl larval stage at twenty-four hours, and every twenty-four hours thereafter, through the late 8pl larva with well-developed rudiment (8-9 days), morphometric measurements were done on 10 larvae from each treatment Measurements were made within 1-1 5 hours on whole mounts.

Following the procedures of McEdward (1984, 1985b) three-dimensional measurements of larvae were collected via computer digitization. Larvae were fixed in 1% formalin in seawater in batches of 2-3 and mounted on slides under coverslips, elevated with plasticene clay, just before beginning the measurements. Microscopic measurements were made under differential interference contrast optics (DIC) using a 10x objective with a total magnification of 156.25x. Measurements included the overall length of the larval body from the posterior tip to the tips of the longest arms (postorals), the midline body length (sensu McEdward, 1984), the lengths of the arms, and the length of the ciliated band The 3-d measurements were made with a digitizing tablet and a rotary encoder, through a custom-built computer interface The horizontal resolution was 0 4 μm and the vertical resolution was 0 6 μm. Data acquisition and morphometric calculations were made using programs written by McEdward.

Skeletal Rod Morphometrics

To visualize the larval skeleton, larvae were dehydrated and cleared After measurements were completed on the larval body, the specimens were removed from the slides and transferred to small petri dishes with 70% ethanol for dehydration Larvae were dehydrated by placing them in two changes of 70% ethanol for five minutes in each change, then transferring them to 100% ethanol for 2-5 minutes. For clearing larvae

were transferred, in a minimum of alcohol, to a small glass dish filled with methyl salicylate. Cleared larvae were placed in a depression slide filled with methyl salicylate, then covered with a coverslip for viewing. For detailed procedures see McEdward and Herrera (in press).

Larval skeletons were visible through the cleared larval tissue. The skeletons were observed using DIC optics and at the same magnification as was used for measurements made on the larval body. Using procedures described in McEdward (1984, 1985b) and McEdward and Herrera (in press), three-dimensional coordinates were digitized for fixed landmark points on the larval skeletal rods. Landmark points were used to calculate the lengths of the major skeletal pieces. These landmark points are the tips and the junctions of the major skeletal rods (Fig. 3).

The skeletal measurements taken were the lengths of the larval arm rods (Fig. 3). Postoral rods were measured from the tips of the rods (Pt. 1,7) to their junctions with the body rods (Pt. 2,8). Anterolateral rods were measured from the tips of the rods (Pt. 6,12) to their junctions with the dorsal-ventral connecting rod (Pt. 5,11). Posterodorsal rods were measured from their tips (Pt. 19,22) to their junctions with the dorsal transverse rods (Pt. 20,23). Preoral rods were measured as the sum of two segments, from their tips (Pt. 16,18) to their junctions with the posterior end of the dorsal arch (Pt. 14). The body rods were measured from their junctions with the postoral rods (Pt. 2,8) to their posterior tips (Pt. 3,9).

Larvae are difficult to see when cleared and smaller stages are easily lost. This fact required the preparation of several more larvae than were used for body

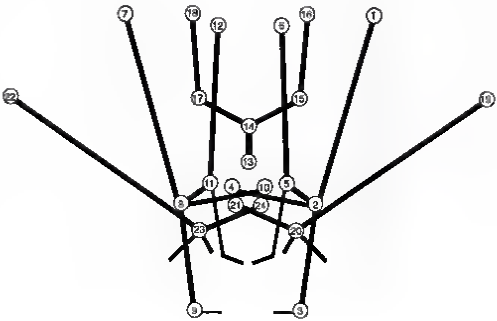


Figure 3 Larval skeletal rods PO rods 1-2 and 7-8 Body rods 2-3, and 8-9, VT rods 2-4 and 8-10, ALA rods 5-6 and 11-12, Dorsal arch 14-15-16 and 14-17-18, PD rods 19-20 and 22-23, DT rods 20-21 and 23-24
(From McEdward & Herrera, in press)

measurements, thus no attempt was made to pair sets of body and skeletal measurements on individual larvae

Statistics

Analyses of the effects of diet and time on the larval body and skeleton included descriptive statistics, two factor ANOVAs with type three sums of squares, and Duncan New Multiple Range Tests. ANOVA's were performed using Statview (Abacus Concepts, Inc., Berkeley, CA, 1992) and SuperANOVA (Abacus Concepts, Inc., Berkeley, CA, 1989) to compare measures of size and shape among stages of development, and among nutritional conditions. A significance level of $p \leq 0.05$ was used for all analyses.

Abbreviations

These abbreviations are used throughout this chapter in the text, table, and figures: PO = postoral, ALA = anterolateral, PD = posterodorsal, PR = preoral; CB = ciliated band, BL = midline body length, BR = body rod, 2pl = 2-armed pluteus, 4pl = 4-armed pluteus, 6pl = 6-armed pluteus; 8pl = 8-armed pluteus; 8R = 8-armed pluteus with juvenile rudiment.

Results

Larval Development

The larvae of *Lytechinus variegatus* developed through metamorphosis within 9 to 11 days at a temperature of 26°C (Table 3, Fig. 4). Larval feeding began by the second day (4pl) as evidenced by the presence of algae in the larval gut. The larvae reached the 6pl stage by the fourth or fifth day and were 8pl larvae by the sixth day. Rudiment formation was visible at 8-9 days of age and larvae could be induced to metamorphose a day or two later.

In the late stage 8pl larvae, specialized locomotory regions of the ciliated band (epaulettes) had developed. These epaulettes formed a transverse ring around the larval body at the base of the arms. A second ring of cilia developed near the posterior end of the larval body (see McEdward & Herrera, in press).

The juvenile rudiment formed at 8-9 days after fertilization. The posterior pedicellaria developed in all the larvae at about day 7 (Fig. 5). Subsequently a second and third pedicellaria appeared on the right side of the body in many larvae. These pedicellariae were located between the two rings of epaulettes. Juvenile skeletal plates could be seen developing at the tips of several larval skeletal rods, particularly at the base of the dorsal arch (Fig. 6) (see McEdward & Herrera, in press).

The skeleton of *L. variegatus* is made up of 6 major elements (Fig. 1, 3). The larval body has a bilateral symmetry, and there are two paired right and left skeletal pieces and two unpaired pieces. The largest of the paired skeletal elements form the body

Table 3. Schedule of larval development in *Lytechinus variegatus*

Day of Age	Stage	Description of Larval Development
1	2pl	PO arms well-developed and extend anteriorly beyond the oral hood. Anterolateral arms absent. Oral hood prominent anterior extension on dorsal side.
2	4pl	Short ALA which extend beyond oral hood. PO arms long and well-developed. Evidence of food in the gut. No body elaboration of ciliated band.
3	4pl	Well-developed 4pl. Both arm pairs well developed. Deepening of PO/ALA armpits and elaboration of ventral transverse ciliated band.
4	4pl	Well-developed 4pl. No evidence of PD arms. Further deepening of PO/ALA armpits and elaboration of ventral transverse ciliated band.
5	6pl	Short stubby PD arms present. First evidence of epaulette formation. Further elaboration of the ciliated band.
6	8pl	PD arms well-developed and about half the length of the PO arms. Short stubby PR arms present. Further development of epaulettes.
7	8pl	All four arm pairs well-developed. Epaulettes well formed. First pedicellaria visible.
8	8R	Early rudiment formation. Variable reductions in the arms of the oral hood. Well developed pedicellaria.
9-10	8R	Well-developed rudiment. Some have three pedicellariae. Beginning of metamorphic competency.

rods, which support the posterior portion of the larval body, the postoral arm rods, and the anterolateral arm rods. There are three additional extensions of these skeletal elements. the ventral transverse rods which support the ventral portion of the larval body, the connecting rods which attach the anterolateral arm rods to the junction of the postoral arm rods and body rods, and the rods that extend posteriorly from the base of the anterolateral arms. Each member of the second pair of skeletal elements is made up of a posterodorsal arm rod and a dorsal transverse rod which supports the dorsal side of the larval body

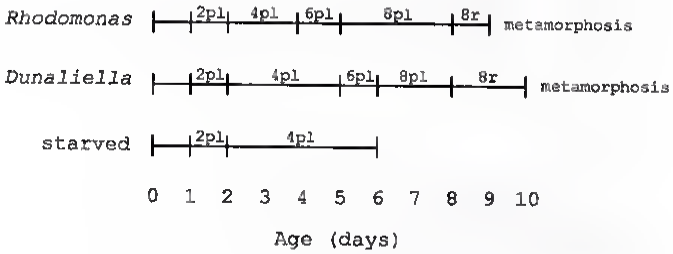
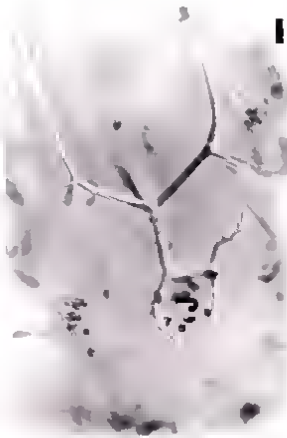


Figure 4 Schedule of larval development and metamorphosis in larvae of the sea urchin *Lytechinus variegatus* at 26°C.

Figure 5 Terminal pedicellaria (P) of *Lytechinus variegatus* Scale bar = 10μm.

p

Figure 6. Base of the dorsal arch of *Lytechinus variegatus* with initial indications of juvenile plate (J) formation. Scale bar = 10μm.



The unpaired skeletal elements are the dorsal arch and a transverse posterior rod. The dorsal arch is a horseshoe shaped skeletal piece consisting of the preoral arm rods which curve around and meet at a junction with a third process, which extends posteriorly along the dorsal midline. The transverse posterior rod is at the extreme posterior end of the larval body and appears to help support this end of the larva. In *L. variegatus*, the larval arm rods are not fenestrated, and the skeleton does not form a "body basket" in the posterior region (for a more extensive discussion of the larval skeleton see McEdward & Herrera, in press)

The paired skeletal elements which form the body rod/postoral arm rod/anterolateral arm rod complex are the first skeletal pieces to form. They are visible at the prism stage. By the end of the first day, the postoral arm rods grow and extend beyond the margin of the larval body to form support for the postoral arms, and the anterolateral arm rods extend to, but not beyond, the anterior edge of the oral hood. The body rods extend to the posterior tip of the body. The ventral transverse rods extend from the postoral/body rod junction and meet at the midline of the body. By day 2 at the 4pl stage the postoral arms have elongated and the anterolateral arm rods have extended beyond the oral hood in formation of the anterolateral arms. On the fourth day, the dorsal arch is visible within the larval body, and on the fifth day the posterodorsal rods can be seen. By the sixth day, the posterodorsal rods extend beyond the larval body to support a pair of posterodorsal arms, and by the seventh day the preoral arm rod extensions of the dorsal arch have grown beyond the margin of the oral hood to form the anterolateral arms. Juvenile plates begin forming and are visible on the eighth day

Larval Morphometry

Larval development time from the 2pl stage to fully developed 8R stage was eight days in the larvae fed 8 cells/ml *Rhodomonas lens* and nine days in larvae fed 8 cells/ml *Dunaliella tertiolecta*. The starved larvae did not develop past the 4pl stage (Fig. 4)

In both fed treatments, larval length increased steadily from the 2pl to the rudiment stage (Fig. 7). The larval length of larvae fed *R. lens* increased from 283 ± 7mm at the 2pl stage to 1252 ± 71mm at the rudiment stage. In larvae fed *D. tertiolecta*, larval length increased from 315 ± 5mm at the 2pl stage to 869 ± 37mm at the rudiment stage. In starved larvae, length increased from 319 ± 6mm at the 2pl stage to 585 ± 15mm on day three (the second day of the 4pl stage) and then decreased to 530 ± 12mm by day six as the larvae deteriorated.

In both fed treatments, body length increased steadily from the 2pl to the rudiment stage (Fig. 8). The body length of larvae fed *R. lens* increased from 194 ± 3mm at the 2pl stage to 760 ± 36mm at the rudiment stage, and in larvae fed *D. tertiolecta* body length increased from 210 ± 3mm at the 2pl stage to 509 ± 19mm at the rudiment stage. In starved larvae body length increased from 216 ± 3mm at the 2pl stage to 306 ± 3mm on day three (the second day of the 4-arm stage) and then decreased to 292 ± 4mm by day six.

The length of the ciliated band (an index of larval feeding capability) increased 12-fold between the 2pl and rudiment stages in larvae fed *R. lens* (Fig. 9). The ciliated band increased only 7-fold in larvae fed *D. tertiolecta*. The length of the ciliated band increased only 2.2-fold in starved larvae. This increase occurred between the 2pl stage

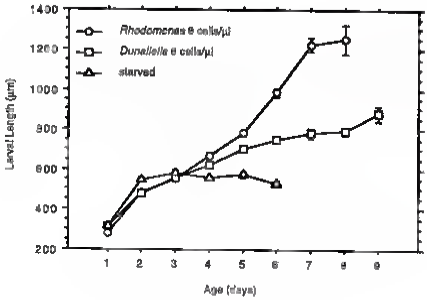


Figure 7 Larval length during larval development of *Lytechinus variegatus*: mean values ± SE.

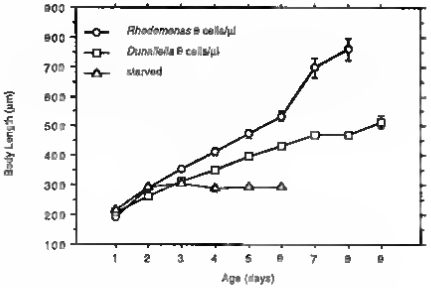


Figure 8 Body length during larval development of *Lytechinus variegatus* mean values ± SE

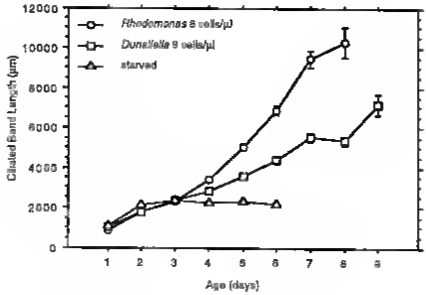


Figure 9 Ciliated band length during larval development of *Lytechinus variegatus*; mean values ± SE

and day three Subsequent to day three the length of the ciliated band decreased in starved larvae.

In larvae fed *R. lens*, the ciliated band length/body length ratio (an index of body shape complexity) increased from 4 38 ± 0 10 at the 2pl stage, to 10 71 ± 0 13 at the 8pl stage, and to 13 60 ± 0 84 at the rudiment stage (Fig. 10). In larvae fed *D. tertiolecta*, the ciliated band length/body length ratio increased from 4 95 ± 0 05 at the 2pl stage, to 11 79 ± 0 34 at the 8pl stage, to 13 87 ± 0 54 at the rudiment stage In starved larvae, this ratio increased from 5 00 ± 0 08 at the 2pl stage (day one) to 7 92 ± 0 40 by day 4 and then dropped to 7 54 ± 0 21 by day six.

The percent ciliated band on the arms increased from day one (2pl) to day two (4pl) in all treatments and then dropped and fluctuated during subsequent development (Fig 11) The percent ciliated band on the arms increased from 60 ± 1 on day one (2pl) to 68 ± 1 on day 2 (4pl) in larvae fed *R. lens*. It increased from 61 ± 1 on day one (2pl) to 73 ± 1 on day two (4pl) in larvae fed *D. tertiolecta*. And, it increased from 61 ± 1 on day one (2pl) to 74 ± 1 on day two (4pl) in starved larvae

In larvae fed *R. lens*, total arm length increased from 257 ± 10mm at the 2pl stage to 3385 ± 294mm at rudiment (Fig. 12) In larvae fed *D. tertiolecta*, total arm length increased from 318 ± 9mm at the 2pl stage to 2315 ± 164mm at rudiment In starved larvae, total arm length increased from 330 ± 11mm on day one (2pl) to a maximum of 854 ± 33mm on day three (second day at 4pl) then decreased as larvae deteriorated

In all larvae, the postoral arms had appeared by the end of the first day In larvae fed *R. lens* the postoral arms were 128 ± 5mm at the 2pl stage The postoral arms

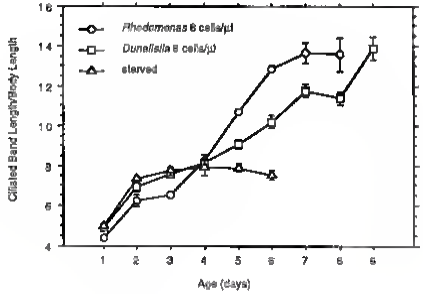


Figure 10 Ciliated band to body length ratio during larval development of *Lytechinus variegatus* mean values ± SE

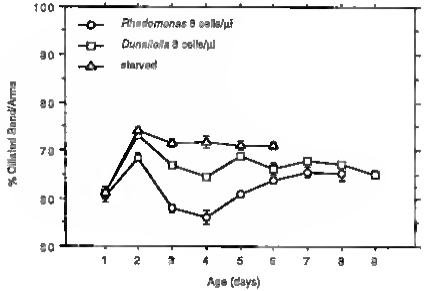


Figure 11 Percent ciliated band on the arms during larval development of *Lytechinus variegatus* mean values ± SE

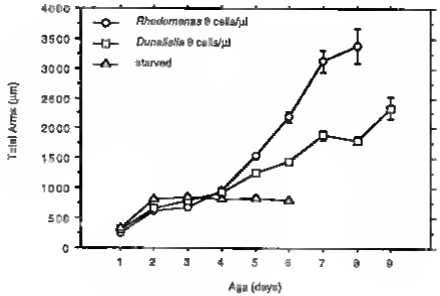


Figure 12 Total arm length during larval development of *Lytechinus variegatus*: mean values ± SE.

reached 474 ± 12mm by the 8pl stage, reached a maximum of 756 ± 15mm on day seven, and then decreased to 703 ± 48mm by the rudiment stage on day eight. In larvae fed *D tertiolecta*, the postoral arms were 159 ± 5mm at the 2pl stage, had reached 500 ± 22mm by the 8pl stage and continued to increase to the rudiment stage reaching 558 ± 21mm. In starved larvae, the postoral arms were 165 ± 5mm at the 2pl stage, reached their maximum length of 343 ± 14mm at day three (4pl) and then decreased as the starved larvae deteriorated. The lengths of the postoral arms on day three for the fed treatments were 276 ± 8mm in those fed *R. lens*, and 308 ± 5mm in those fed *D tertiolecta*.

The anterolateral arms appeared by day two in all larvae. In larvae fed *R. lens*, the anterolateral arms were 64 ± 4mm at the 4pl stage (day two), had increased to 89 ± 15mm at the 8pl stage (day five), and were 286 ± 80mm by the rudiment stage (day eight). In larvae fed *D tertiolecta*, the anterolateral arms were 71 ± 6mm at the 4pl stage (day two), had increased to 165 ± 22mm at the 8pl stage (day seven), there was no significant increase in length in this arm pair after day six. In starved larvae the anterolateral arms were 78 ± 4mm at the 4pl stage (day two) and had increased to 102 ± 5mm by the sixth day. Unlike the postoral arms, the anterolateral arms continued to increase in length and did not appear to deteriorate from the third to the sixth day.

The posterodorsal arm pair appeared at day four in larvae fed *R. lens*. These arms were 63 ± 28mm at day four, 166 ± 7mm at the 8-arm stage (day five) and had reached 522 ± 67mm by the rudiment stage. In larvae fed *D tertiolecta*, the posterodorsal arms appeared at day 5, and were 67 ± 4mm on day five, increased to 221 ± 15mm at the

8-arm stage (day seven), and then to 334 ± 19mm by the rudiment stage. Posterodorsal arms did not develop in starved larvae.

The final arm pair, the preoral arms, formed on day five in the larvae fed *R. lens* They were 49 ± 3mm in length on day five in these larvae and had increased in length to 182 ± 44mm by the rudiment stage (day eight). In larvae fed *D. tertiolecta*, the preoral arms were 60 ± 6mm at the 8pl stage (day seven) and had increased to 107 ± 19mm by the rudiment stage. These arms appeared to be growing faster in the larvae fed *R. lens*. In larvae fed *R. lens*, the preoral arms increased from 49 ± 3mm on day five to 172 ± 19mm on day seven, compared to an increase from 60 ± 6mm on day seven to 107 ± 19mm on day nine in larvae fed *D. tertiolecta*. Starved larvae did not develop preoral arms.

In all larvae, the postoral arm rods, anterolateral arm rods, ventral transverse rods and body rods had appeared by the end of the first day (Table 4). In larvae fed *R. lens*, the postoral arm rods were 152 ± 8mm at the 2pl stage (day 1) (Fig. 13). The postoral arm rods reached 591 ± 9mm by the 8pl stage (day 5), and were 867 ± 12mm by the late 8pl stage (day seven). In larvae fed *D. tertiolecta*, the postoral arm rods were 172 ± 6mm at the 2pl stage (day 1), had reached 585 ± 23mm by the 8pl stage (day 7) and continued to increase to the rudiment stage (day 9) reaching 660 ± 26mm. In starved larvae the postoral arm rods were 196 ± 3mm at the 2pl stage (day 1), reached their maximum length of 461 ± 11mm at day three (4pl) and then decreased to 393 ± 13mm by day six as the starved larvae deteriorated. The lengths of the postoral arm rods on day three for the fed treatments were 395 mm in both fed treatments (± 10mm in the *R. lens* treatment, ± 6mm in the *D. tertiolecta* treatment).

The anterolateral arms appeared by day two in all larvae. In larvae fed *R. lens*, the anterolateral arm rods were 187 ± 4mm at the 4pl stage (day two). The anterolateral arm rods reached 392 ± 27mm by the 8pl stage (day 5), and were 575 ± 44mm by the late 8pl stage (day seven). In larvae fed *D. tertiolecta*, the anterolateral arm rods were 168 ± 8mm at the 4pl stage (dsy two), had reached 404 ±18mm by the 8pl stage (dsy 7) and

Table 4. Schedule of larval skeleton development in *Lytechinus variegatus*.

Day of Age	Stage	Description of Larval Development
1	2pl	A single pair of multirod skeletal elements present. PO rods extend into the PO arms. ALA rods extend to tip of oral hood. Body rods are simple and extend to posterior of body. A simple pair of recurrent rods present and the ventral transverse rods meet at the midline of the body
2	4pl	ALA rods extend beyond the oral hood into short ALA arms. Recurrent rods develop posterior transverse processes. Ventral transverse rods cross near sagittal midline. Body rod has developed transverse processes and projections
3	4pl	Well developed 4pl. Both arm pairs well developed. Dorsal arch present in some as small triradiate spicule
4	4pl	Posterior extensions of recurrent rods cross at midline. Dorsal arch appears in all, some with elongation of anterior components toward the tip of oral hood. PD rods appear as small triradiate spicules near the junctions of the PO and body rods.
5	6pl	PD rods extend into short PD arms. Dorsal arch continues anterior development and develops dorsally directed spurs which will support a ciliated lobe on the dorsal side of oral hood.
6	8pl	Dorsal arch extends beyond the oral hood to form preoral arms. Continued elongation of PD, PO and ALA rods. Recurrent rods regressing
7	8pl	First pedicellaria in some. Partial regression of left ALA rod. Base of dorsal arch forming ring of juvenile skeleton
8	8R	Juvenile skeleton developing. Many of the left pair of larval skeletal rods regressed
9-10	8R	Well-developed rudiment. Some have three pedicellariae. Degeneration of larval skeleton continues. Beginning of metamorphic competency

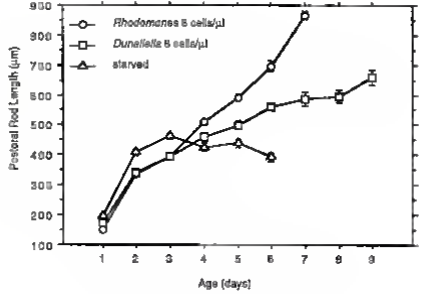


Figure 13 Larval skeletal dimensions length of postoral rods during larval development of *Lytechinus variegatus* mean values ± SE

continued to increase to the rudiment stage (day 9) reaching 477 ± 42mm (Fig. 14). In starved larvae, the anterolateral arm rods reached their maximum length of 246 ± 9mm at day three (4pl) and there was no significant difference in anterolateral arm rod length between day three and day six (223 ± 18mm). The lengths of the anterolateral arm rods on day three for the fed treatments were 233 ± 9mm in those fed *R. lens*, and 234 ± 4mm in those fed *D. tertiolecta*.

In larvae fed *R. lens*, the ventral transverse rods were 57 ± 2mm at the 2pl stage (day 1), reached 144 ± 7mm by the 8pl stage (day 5), and were 231 ± 7mm by the late 8pl stage (day seven). In larvae fed *D. tertiolecta*, the ventral transverse rods were 56 ± 2mm at the 2pl stage (day 1), had reached 153 ± 12mm by the 8pl stage (day 7) and continued to increase to the rudiment stage (day 9) reaching 198 ± 15mm (Fig. 15). In starved larvae, the ventral transverse rods were 53 ± 2mm at the 2pl stage (day 1), reached their maximum length of 89 ± 3mm at day four (4pl) and there was no significant difference in ventral transverse rod length between day four and day six.

In larvae fed *R. lens*, the body rods were 102 ± 3mm at the 2pl stage (day 1), and there was no significant change in their length until day seven at which time their length had decreased to 88 ± 4mm (Fig. 16). There was no significant difference in body rod length between any age classes in larvae fed *D. tertiolecta*. There was no significant difference in body rod length between day one and day six (when measurements were stopped) in starved larvae.

The posterodorsal arms and their rods appeared at day four in larvae fed *R. lens* (Table 4). These arm rods were 72 ± 8mm at day four, 211 ± 7mm at the 8pl stage (day

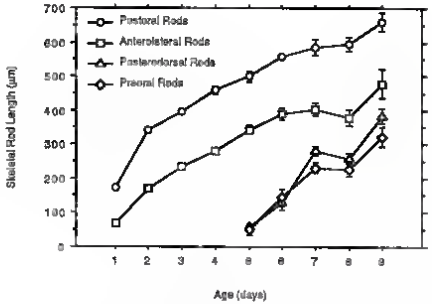


Figure 14 Larval skeletal dimensions during larval development of *Lytechinus variegatus* in larvae fed 8 cells µl^{-1} of *Dunaliella tertiolecta*: lengths of the arm rods for the postorals, anterolaterals, posterodorsals, and preorals, mean values ± SE.

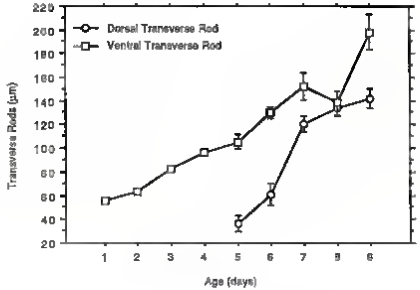


Figure 15. Larval skeletal dimensions during larval development of *Lytechinus variegatus* in larvae fed 8 cells µl^{-1} of *Dunaliella tertiolecta* skeletal rod lengths of the dorsal transverse and ventral transverse rods, mean values ± SE.

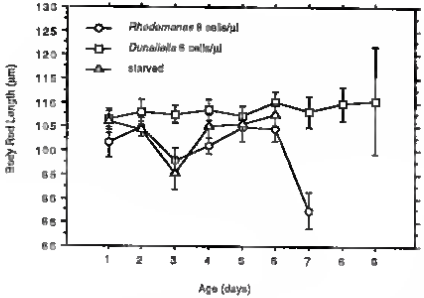


Figure 16. Larval skeletal dimensions length of body rods during development of *Lytechinus variegatus* mean values ± SE.

five) and had reached 553 ± 19mm by day seven when measurements were terminated. In larvae fed *D. tertiolecta*, the posterodorsal arms and their rods appeared at day five and the rods were 57 ± 9mm on that day, increased to 280 ± 15mm at the 8pl stage (day seven), and then to 384 ± 21mm by day nine (Fig. 14). Posterodorsal arms and their rods did not develop in starved larvae.

The dorsal transverse rods appeared at day four in larvae fed *R. lens* (Table 4). These rods were 38 ± 5mm at day four and had reached 178 ± 8mm by day seven when measurements were terminated. In larvae fed *D. tertiolecta*, the dorsal transverse rods appeared at day 5 and were 36 ± 7mm on that day. They increased to 142 ± 8mm by day nine (Fig. 15). Dorsal transverse rods did not develop in starved larvae.

The final arm pair, the preoral arms, formed on day five in the larvae fed *R. lens*, but the preoral arm rods formed on day three (Table 4) and they were 12 ± 3mm long on day three. They had increased to 405 ± 23mm by day seven. In larvae fed *D. tertiolecta*, the preoral arm appeared on day seven but the preoral arm rods appeared on day five and they were 50 ± 17mm long on day five (Fig. 14). They increased in length to 232 ± 15mm by day seven and to 323 ± 28mm by day nine. Starved larvae did not develop preoral arms.

Discussion

Larval Development in *Lytechinus variegatus*

A general description of larval development, and a developmental schedule from fertilization to metamorphosis (at 25-27°C), for *Lytechinus variegatus* was presented in

Chapter 2 The results obtained here differ little from those obtained in the previous study when compared at similar levels of food

In later stage larvae, there were several sites where locomotory cilia developed from the ciliated band. There were two posterior epaulette rings. One, as described by Mazur and Miller (1971), was a telotrochal ring, and the other formed as a ring around the body near the bases of the postoral and posterodorsal arms. Mortensen (1921) reported an area of locomotory cilia in the posterior region but described it as posterolateral processes rather than a ring epaulette In addition to the two epaulette rings there are several locomotory lobes of the ciliated band on either side of the bases of the postoral and posterodorsal arms There are also other lobes on the larval body (particularly on the dorsal side) that have locomotory cilia.

Morphometric Description of Larval Development in *L. variegatus*

Larvae fed 8 cells μl^{-1} of *D. tertiolecta* reached metamorphic competency by day 11 Three morphometric measurements of pluteus size were made The larval length, from the tip of the postoral arms to the posterior tip of the body, of these plutei increased approximately 3-fold from the 2pl to the 8R stage (Fig. 7) The body length, measured from the midline of the anterior tip of the oral hood to the posterior tip of the body, increased approximately 2.5-fold from the 2pl to the 8R stage (Fig. 8) The length of the larval feeding structure, the ciliated band, increased approximately 7.25-fold from the 2pl to the 8R stage (Fig. 9)

The ratio of the ciliated band length to the body length is an indicator of larval shape This ratio increased from 4 95 at the 2pl stage to 13 87 at the 8R (Fig 10) Thus

ratio indicates that the ciliated band length increased relative to body length and that this increase is due to changes in larval shape rather than just increases in body size Isometric growth accounts for 17% of the increase in the ciliated band, while allometric growth accounts for 83% of the increase Another indicator of larval shape change is the percentage of ciliated band found on the arm pairs. At the 2pl stage the postoral arms, of course account for 100% of the ciliated band on the arms. This percentage decreases as each new pair of arms is added until it drops to 49% at day 9, the 8R stage (Fig. 11) The anterolateral arms contribute about 22% to the ciliated band on the arms at the 4pl stage and this percentage also drops to about 12% as the other two pairs of arms are added. The posterodorsal arms account for about 11% of the ciliated band on the arms at the 6pl stage and this percentage increases to 30% at day 9 The preoral arms account for 6-9% of the ciliated band on the arms during the last few days of development.

Effects of Food Type (*Rhodomonas lens* vs. *Dunaliella tertiolecta*)

Larvae fed 8 cells μl^{-1} of *Rhodomonas lens* reached metamorphic competency (9days post fertilization) sooner than did those fed 8 cells μl^{-1} of *Dunaliella tertiolecta* (11days post fertilization) Larvae from both treatments were of similar size until the fifth day (6-armed stage) when those fed *R. lens* attained a larger larval size in every measurement taken. Total larval length (Fig. 7) and midline body length (Fig. 8) through day 5 were similar for larvae raised on either diet After the fifth day, both of these measures of larval size diverged substantially between diets, and by the 8R stage, the larvae fed *R. lens* were approximately 50% longer than those fed *D. tertiolecta* From day 5 (6pl stage) throughout the rest of development, the larval arms and their skeletal rods were longer in larvae fed *R. lens* (Fig. 12, 13) The ciliated band grew much longer

in those larvae that were fed *R. lens*. Larval feeding ability is directly proportional to band length.

The ciliated band length to body length ratio is an indicator of larval shape change. It denotes the amount of growth of the larval feeding apparatus, the ciliated band, relative to the size of the larval body. At the 8R stage the larvae that were fed *R. lens* did not have a more complex shape than those fed *D. tertiolecta* (Fig. 10). The larvae fed *R. lens* did have longer ciliated bands and were longer in all measurements of size, but their increased capacity to feed was a result of these size changes and not a result of any development of a more complex shape. The percentage of ciliated band on the arms (Fig. 11) is also an indicator of shape change. The differences, seen between larvae fed these two diets, in this parameter were small and varied little between or within the treatments. These data suggest that differences in the nutritional quality of the algal species offered as food determines the rate at which larvae grow and develop, but it does not affect the trajectory of development.

The larvae fed *R. lens* had longer ciliated bands, but they were also larger in measures of larval size, and the ratio of their ciliated band length to body length did not differ from that of the larvae fed *D. tertiolecta*. The larvae fed *R. lens* were able to capture more cells, but they were also larger. The ability to feed relative to body size was not different between these nutritional treatments. The difference in body size may have been genetic because the nutritional treatments were not applied to larvae from the same parents.

Starvation and Morphological Plasticity

Starved larvae did not reach metamorphic competency (Fig. 4). The larvae of *L. variegatus* can develop to only the 4pl stage without exogenous particulate food. No additional arm pairs developed, and the total length of the arms did not increase after day 2 (Fig. 12, 13). The larvae survived for at least 6 days, but they stopped growing and developing after day 2 (Fig. 4, 8, 9), and in many, the arm rods of the skeleton began to protrude beyond the soft tissues at the tips of the arms.

Ciliated band lengths were the same in both starved and fed treatments on day 1 at the 2pl stage. The length of the ciliated band in the fed larvae increased steadily from day 1 to day 6, while that of the starved larvae increased from day 1 to day 2 and did not increase significantly after that. In starved larvae, there was no change in larval shape after day 2 as evidenced by the fact that the ciliated band length to body length ratio did not increase (Fig. 10). This ratio increased rapidly from day 1 to day 2 for both treatments but there were no significant differences in the ratios until day 5, when fed larvae reached the 6pl stage and continued to increase the ratio of ciliated band length to body length. Starved larvae plateau after the second day (4pl) as they fail to form the last 2 pairs of arms and begin to deteriorate. *Lytechinus variegatus* has a very clear need to feed early in its larval life. It is an early obligate planktotroph (sensu Herrera *et al.*, 1996; McEdward, 1997). *Lytechinus variegatus* depends on particulate feeding very early in larval life. These larvae have a limited capacity for development beyond the early larval stages, without feeding. This is similar to some of the starved larvae from previously studied species (Boidron-Metairon, 1988; Fenaux *et al*., 1988) but is in striking contrast

to other subtropical echinoids with higher levels of endogenous reserves (Eckert, 1995, Herrera *et al*, 1996).

Starvation did not affect the length of the body rod, which forms early in larval life (by the 2-armed stage) and does not grow later in development but did influence the length of the arm rods. Early skeletal formation is not influenced by exogenous food supply, but later growth is affected by environmental conditions and is subject to variation in response to food levels (Boidron-Metairon, 1988, Hart & Scheibling, 1988; Strathmann *et al*, 1992, Fenaux *et al*, 1994).

In starved larvae of *L. variegatus* at day 2, the arms were longer than those of fed sibling larvae (Fig. 12). Also, the overall body length, a measurement of larval size which includes the length of the postoral arms, the total arm length, and the length of the ciliated band of starved larvae were significantly longer than those of fed sibling larvae on day 2 (Fig. 8). This may indicate a plastic response to the absence of food similar to that seen in late stage larvae experiencing starvation due to low levels of food (Boidron-Metairon, 1988; Strathmann *et al*., 1992, Fenaux *et al*., 1994). Larvae grow longer arms in response to low levels of food. This allows them to gather more exogenous resources to fuel their development. This response appears to be more evident in strongly obligate planktotrophs (present study) than in larvae from species with larger eggs, a longer facultative period, and less dependence on exogenous food. Eckert (1995) did not find evidence of plasticity in larvae of the sand dollar *Encope michelini*, an echinoid with an egg size of 175 µm. *L. variegatus* has a much smaller egg size of 110µm (Mazur & Miller, 1971, Chapter 4).

Development and Growth of the Larval Skeleton

The skeleton of the pluteus of *L. variegatus* is composed of 6 skeletal pieces (Fig 3). These skeletal pieces are the main skeletal elements which are characteristic of the echinoid crown-group ancestor (Wray, 1992). The major difference in the *L. variegatus* skeleton is that the postoral and posterodorsal arm rods are not fenestrated. Also of interest, and reported by Mortensen (1921), is the fact at *L. variegatus* does not form a skeletal body basket in the posterior region of the body. Most of the larvae developed a terminal pedicellaria at the posterior tip of the body. Later, many also developed two lateral pedicellariae on the right side of the body, between the two epaulette rings.

Measurements revealed that the length of the body rod does not increase during development from the 2pl through the 8pl stage (Fig. 16). This indicates that during pluteus development, the larva increases its length in an anterior direction, with most of the increase in length attributable to the growth of the arms.

In larvae fed *D. tertiolecta*, the postoral arms increase in length from approximately 160µm to about 560µm. This increase is mirrored in measurements of the length of the postoral skeletal rods which increased from approximately 170µm to 660µm (Fig. 13). The skeletal rods are longer than the arms, because the bases of the skeletal rods are within the body (Fig. 1).

The length of the second pair of arms, the anterolaterals, does not increase as much as the length of the postorals. They increase in length from approximately 70µm at 2 days of age (early 4pl) to about 160µm at 9 days. The anterolateral arm rods increase from about 170µm to 480µm during the same time period (Fig. 13). The length of the anterolateral skeletal rods does not follow the length of the anterolateral arms as closely

as the postoral rods follow the postoral arms, because much of their length is within the oral hood, with only their distal tips supporting the anterolateral arms (Fig. 1).

The posterodorsal arms increase in length from about 70μm to 340μm and only reach approximately 60% of the length of the postoral arms. Mortensen (1921) also reported that the posterodorsal arms of *L. variegatus* are shorter than the postoral arms. The posterodorsal arm rods increased from about 60μm to 380μm (Fig. 13).

The preoral arms grew from about 60μm to 110μm and their skeletal rods did not closely follow their growth because much of the dorsal arch is within the oral hood and the dorsal arch begins forming long before the preoral arms are visible. The preoral arm rods increased from 10μm to 400μm during development (Fig. 13).

Comparisons of Growth and Form among Four Species

Morphometric studies of larval growth and development have been done on three other species of urchins. All of these species *Dendraster excentricus*, *Strongylocentrotus purpuratus* and *Strongylocentrotus droebachiensis* (McEdward, 1984, 1986a, b) are cold temperate urchins. In each of these studies, as in the present one, larvae were reared on high concentrations of *D. tertiolecta* and three-dimensional morphometric measurements were done using the same techniques (McEdward, 1984). Comparisons may help identify what aspects of larval growth and development are common to most sea urchins, perhaps illuminate the diversification of echinoid larvae, and will provide a basis for future studies across taxa within the echinoids.

Differences in larval development among these urchin species may be caused by genetics, egg size, and/or environmental conditions such as temperature. Three of the

urchins (*Lytechinus* and the two strongylocentrotids) are in the order Echinoida and one (*Dendraster*) is in the order Clypeasteroidea (Smith, 1984). Development rate, larval size and larval shape are related to egg size (Sinervo & McEdward, 1988; Herrera *et al.*, 1996). The diameters of the eggs of each species are *S. purpuratus* 80µm, *L. variegatus* 110 µm, *D. excentricus* 125µm, and *S. droebachiensis* 150µm (Mazur & Miller, 1971; McEdward, 1986a, Chapter 4). *Lytechinus variegatus* is a tropical/subtropical echinoid from the Gulf of Mexico and Caribbean. Differences in development between *L. variegatus* and the other three species could be due to temperature or seasonality between their native locales.

Development time

Total development time is influenced by temperature (McEdward, 1985a). *L. variegatus* developed much more rapidly than did the other three species (McEdward, 1986b). This difference may be due to environmental conditions rather than phylogeny or egg size. There are five phases in the larval development of echinoplutei. The first phase includes the period from fertilization until the larva is able to feed (usually the 2pl or 4pl stage). The second phase is the period of arm formation from the 2pl to the fully formed 8pl stage. After the 8-armed body form is attained, there is a period of arm elongation (sensu McEdward, 1984). This is the third phase. The fourth phase is the period during which the juvenile rudiment is completed and metamorphic competency is attained. The larval arms continue to elongate during the fourth phase. Finally, there is a fifth phase during which larvae are competent to metamorphose but may delay metamorphosis. During the final phase, the juvenile rudiment may increase in size (Chapter 2). The first two phases of larval development combined may be considered to

be the period of formation of the larval body, as in the work of McEdward (1984). The duration of the period of larval body formation compared to the duration of the time of rudiment formation is very similar in all of these species with the exception of *S. droebachiensis* (McEdward & Herrera, in press). In the other three species the period of larval body formation is 58-66% of the total time from fertilization to formation of the rudiment. In *S. droebachiensis* relative time to form the rudiment is very rapid, and the formation of the larval body comprises 83% of the time from fertilization to metamorphic competency. This difference is probably due to the large egg size found in this species, providing more endogenous reserves and an increased capability for acquiring reserves via facultative feeding (Chapter 4), for the relatively rapid formation of the rudiment.

Larval size

The larvae of the clypeasteroid *D. excentricus* are smaller than the larvae of the other three species. Sand dollars often have smaller larvae with less elaborate bodies lacking the body lobes and attendant convolutions of the ciliated band seen in *L. variegatus* larvae (Chapter 4). Ciliated band length and total length of the arms are similar between *D. excentricus* and *L. variegatus*. They are also similar between the two strongylocentrotids but differ between the two groups. This difference is not related to egg size or an environmental condition such as temperature but could illustrate a feature that is specific to the strongylocentrotids (McEdward & Herrera, in press). An alternative hypothesis is that a larger larval size and longer ciliated band and total arm lengths may all be characteristic of the Echinoidu, but not all of these characteristics are preserved in *L. variegatus* because growth of the larval body, including arm growth which affects ciliated band length, appears to slow significantly during rudiment formation in this

species. This phenomenon does not appear to be related to geography as it is not apparent in other subtropical species (pers. obs.) but more work is needed on other subtropical members of the Echinoida.

Larval shape

The ciliated band to body length ratio, an indicator of body shape, is higher in larvae *of L. variegatus* throughout development of the larval body, indicating that these larvae have a more complex shape than the larvae of the other three species. This difference may be due to phylogeny. *Lytechinus variegatus* is a toxopneustid, a group which is characterized by a number of elaborate lobes which increase the length of the ciliated band. This group also has an exceptionally wide preoral transverse band (Wray, 1992).

Another indication of larval shape is the percentage of ciliated band on the arms. From the 4pl through the 8R stage all four species had similar percentages of ciliated band found on the arms (60-70%). This percentage may be a common feature among species. In the strongylocentrotids there are no data on which arms account for most of the elaboration of the ciliated band, but data on the other two species show that the arms which are responsible for most of the ciliated band, and thus the feeding capability of the larvae, differ between *L. variegatus* and *D. excentricus*. In *D. excentricus* the posterodorsals are the longest arm at the 8R stage, while in *L. variegatus* the postorals contribute most to the length of the ciliated band. This difference may indicate a fundamental design difference between larvae of the Echinoida and the Clypeasteroida. However, *L. variegatus* is known to have unusually short posterodoral arms in comparison to the length of the postoral arms and thus may be a family or species

characteristic rather than a feature common to the entire order (Mortensen, 1921). In addition, there appears to be a major difference between the Echinoida and the Clypeasteroida in that the larval arms of the latter seem to grow rapidly between the 8-armed and 8-armed/with rudiment stages, while the growth of the larval arms in the Echinoida slows at the final larval stages (McEdward, 1984, McEdward & Herrera, in press, Chapter 5, personal observations). All four species had similar increases in ciliated band length to body length ratios during overall development, but larvae of *D. excentricus* experienced a large proportion of that increase during rudiment formation due to continued rapid elongation of the postoral and posterodorsal arms (McEdward, 1984).

Allometry of larval growth

In echinoplutei, the ability to capture food is limited by the length of the ciliated band (Strathmann, 1971, Strathmann *et al*., 1972). This feeding structure is linear while the body of the larva grows in three dimensions. In order to increase feeding ability the larva must grow a longer ciliated band in relation to its body length. Changes in larval body shape allow positive allometric growth of the ciliated band. In this way larvae are able to increase their linear feeding structure in proportion to cubic increases in body size (McEdward, 1984). These species comparisons show that in all four species studied to date, a similar amount (between 76 - 86%) of the increase in ciliated band length is due to larval shape changes. Allometric growth is a common characteristic of echinoplutei (McEdward & Herrera, in press).

During the formation of the rudiment, larval arm pairs grow at different rates relative to the body of the larva. In *D. excentricus* the postoral and especially the

posterodorsal arms show strong allometric growth and account for most of the shape changes that occur during the 8-armed stage (8pl-8R) (McEdward, 1984). *L. variegatus* larvae also continue to change shape during the 8-armed stage. All of the arms, except the anterolaterals, increase in length during the 8-armed stage. The postoral arms show positive allometric growth of 32% (McEdward & Herrera, in press). The posterodorsal and preoral arms accounted for most of the shape change with percentages of allometric growth of 84% and 88% (McEdward & Herrera, in press) Although different arm pairs account for most of the increase in the ciliated band between these two species, they both demonstrate the same general pattern of larval shape changes which result in increased feeding ability and allow a linear structure to support body growth.

These findings on larval growth and development in *L. variegatus*, in addition to previous studies of three other species illustrate some common features of growth and form in echinoid larvae, regardless of phylogeny or geography One important feature is that the ciliated band feeding structure grows relative to body size in a similar way and by similar means. The length of the ciliated band increases relative to the length of the body throughout development through all of the larval stages. This is accomplished by the development and elongation of four pairs of larval arms. In each of the four species compared, there is a similar percentage of the ciliated band feeding structure found on the larval arms There might be interordinal differences in which pairs of arms account for most of the change in shape, the increase in length of the ciliated band, and the timing of elongation of the larval arms The Clypeasteroida grow more during the period of rudiment formation than do the Echinoida. The Echinoida complete most of their growth during the phases of larval arm formation.

Larvae of the subtropical echinoid *L. variegatus* differ from the three cold temperate species in that they progress very rapidly through development (9-11 days), and have a more complex body shape throughout their development with many elaborate ciliated lobes. Another difference is that *L. variegatus* larvae exhibit very little growth during the formation of the rudiment. The comparison of these studies illuminates some of the common features of and the differences in larval growth in echinoplutei.

This study revealed that differences in exogenous energy sources affect the time required to reach metamorphosis, as well as influencing the morphology of the larval body. Larvae fed *R. lens* had shorter arms at each stage during larval development (except 8R) and reached metamorphosis sooner than did larvae fed *D. tertiolecta*. Starved larvae had longer postoral arms at the 2pl stage and longer ALA arms at the 4pl stage than did fed larvae. These findings support the hypothesis that high levels of exogenous food can have effects on larval morphology similar to those from increases in endogenous reserves, and might mimic an evolutionary increase in egg size (Strathmann *et al.*, 1992).

Allometric increases in feeding structures are also characteristic of molluscan veligers (Jespersen & Olsen, 1982). Plastic responses to limited food have also been observed in these larvae (Strathmann *et al.*, 1993; Padilla, pers. comm.). While echinoplutei increase the length of the ciliated band to increase feeding capability, veligers increase both the length of the ciliated band and the length of the prototrochal cilia (Strathmann *et al.*, 1993). Veligers are able to increase feeding by increasing the length of these cilia because they catch particles using a downstream ciliary stroke, rather than by reversal of ciliary beat (Strathmann, *et al.* 1972).

Cyphonautes larvae of bryozoans also have a feeding structure similar to that of echinoplutei, in that it is a single band but they do not feed on downstream particles using a localized reversal of ciliary beat (Strathmann & McEdward, 1986). In cyphonautes the length of the feeding structure increases isometrically in relation to their body size, and thus have low clearance rates for their size (McEdward & Strathmann, 1987). Their metabolic capacity is similar to that of echinoplutei, as is the protein content of advanced stages (McEdward & Strathmann, 1987). No mechanism has been discovered by which they might be able to increase their intake of food particles. Their development time is not thought to be extraordinarily long (McEdward & Strathmann, 1987). It is possible that cyphonautes do not require as much energy to reach metamorphosis as do echinoplutei.

Similarities and differences among echinoplutei from different regions, among the echinoplutei of the Echinoida and Clypeasteroida, among larvae from diverse taxa with similar feeding mechanisms, and those with different feeding mechanisms, are functionally important. More work is needed among these taxa, and others, within a phylogenetic framework to determine the generality of these results and the implications of these findings to the study of larval ecology and life history evolution.

CHAPTER 4
DIVERSITY OF NUTRITIONAL STRATEGIES AMONG ECHINOID LARVAE AND THE TRANSITION FROM FEEDING TO NONFEEDING DEVELOPMENT

Introduction

Historical Background

In the past, there have been two commonly recognized and contrasting types of pelagic larval development planktotrophy (feeding) and lecithotrophy (nonfeeding) in the approximately 30 phyla of marine benthic invertebrates (Thorson, 1950, Mileikovsky, 1971, Chia, 1974, Grahame & Branch, 1985; Jablonski & Lutz, 1983, Levin & Bridges, 1995) Species with planktotrophic development were thought to partition reproductive resources into many small eggs, with a relatively minimum investment in energy, to produce small larvae with elaborate feeding structures and an extreme and early dependence on feeding. These larvae were thought to spend a relatively long pelagic period feeding on phytoplankton. These species would gain the advantage of increased fecundity and the ability to use larval food sources in the plankton (Strathmann, 1985, Rumrill, 1990, Wray & Raff, 1991)

Species with pelagic lecithotrophic development produce fewer eggs per unit of energy devoted to reproduction. Species with lecithotrophic development were characterized by large yolky larvae which lack feeding structures, spend a shorter time in

the plankton, and are not able to feed (for review see Day & McEdward, 1984; Levin & Bridges, 1995)

These developmental modes, planktotrophy (feeding) and lecithotrophy (nonfeeding) are correlated with egg size. Eggs that develop into feeding larvae are smaller and contain ~1000 times less energy than eggs that develop into nonfeeding larvae (Strathmann & Vedder, 1977, Turner & Lawrence, 1979, McClintock & Pearse,1986, McEdward, 1991, McEdward & Chia, 1991, Eckelbarger, 1994) For example, in echinoid and asteroid echinoderms, small eggs (60-220μm diameter = 0 11-5.58μl volume) contain 1 139-9.503x10^{-3} joules egg^{-1} (McEdward, 1991) and develop into feeding larvae Larger eggs (>350μm diameter > 22 45μl volume) contain 2.6 joules egg^{-1} (McEdward, 1991) and develop into nonfeeding larvae (Emlet *et al* , 1987, Emlet, 1990). In species which free spawn, and do not provide parental care, the contents of the egg are the entire maternal investment In these species, it is assumed that egg energy content determines fitness by influencing larval traits that affect survival during the pelagic period (e g., Vance, 1973a, b; Christiansen and Fenchel, 1979, Strathmann, 1985, Havenhand, 1995 McEdward, 1997)

Many marine invertebrate phyla appear to have evolved with a planktonic feeding larva in place (Jägersten, 1972, Strathmann, R.R., 1978b, 1987, 1993) This is the most parsimonious explanation for the sharing of the single band feeding structure in the enteropneust hemichordates, echinoderms, and the lophophorate phyla (Strathmann, 1978b, 1987, 1993) Thus, planktonic feeding larvae would seem to be the pleisiomorphic (ancestral) condition in these phyla. Nonfeeding larvae have evolved in many taxa (Strathmann, 1978b 1993, Ghiselin, 1987, Bridges, 1993 Wray, 1995, Levin & Bridges

1995) It has been suggested that when larval food sources are scarce it is advantageous for species to produce nonfeeding larvae, and when food is abundant it is advantageous to have feeding larval forms (Vance, 1973a, b; Strathmann, 1978b, Wray & Raff, 1991) The basic feeding larval form is morphologically complex and exhibits some structural change with changes in egg size (e.g., Sinervo & McEdward, 1988), but only undergoes extensive morphological simplification during the transition to a nonfeeding larval form (Strathmann, R.R., 1987; Wray & Raff, 1991) The presence of vestigial structures in some nonfeeding larval forms is evidence that nonfeeding forms have evolved from feeding forms (Okazaki, 1975, Hendler, 1982, Raff *et al*, 1987; Parks *et al*, 1989; Amemiya & Emlet, 1992, Olson *et al.*, 1993)

Traditional Life History Models

The advantages and disadvantages of producing a large number of small, energetically inexpensive eggs versus those of producing a few large, expensive eggs has been repeatedly modeled and analyzed in an attempt to understand the marine invertebrate reproductive strategies we see in nature (Vance, 1973a, b, Strathmann, 1977, Christiansen & Fenchel, 1979; Roughgarden, 1989, see review by Havenhand, 1995) Vance's model (1973a, b) provides an illustration of the main theme of these life history models. His model examined the relationships among egg size, planktonic mortality, and development time. According to his model, each egg size would have a different "reproductive efficiency", which he defined as the number of larvae which settle and metamorphose per unit of reproductive energy Because of the energetic costs of reproduction, higher

reproductive efficiency should correspond to greater fitness and thus be favored by selection.

Vance's model viewed larval development as two successive stages: prefeeding (i.e., fueled by reserves in the egg) and feeding (i.e., dependent on exogenous food) (Fig. 17). The feeding period for planktotrophic larvae begins as soon as the feeding structures develop. Lecithotrophic larvae can't feed until after the end of larval development. The duration of their larval feeding stage is zero (Fig. 17). Thus, planktotrophy and lecithotrophy can be defined as extremes in the timing of the developmental transition between the prefeeding and feeding stages.

The energy content of the egg [s] is the proportion of the amount of energy required for development to metamorphosis. The value of s is defined over the range from 0 to 1. An egg size with a value of 1.0 provides the larva with enough energy to reach metamorphosis without needing to feed. This is lecithotrophy. All egg sizes with $s < 1.0$ do not have sufficient energy to complete development to metamorphosis, and require some feeding. This suggests that there could be a range of egg sizes and energetic strategies.

Examining the parameters of predation, fecundity, and development time, Vance's model predicted that maximum reproductive efficiencies were for the extremes of the range $0<s<1$. Thus, the two extremes in reproductive strategy, completely feeding (planktotrophic) or completely nonfeeding (lecithotrophic) development are predicted to be favored by selection. An egg size of intermediate energetic content would be selected against.

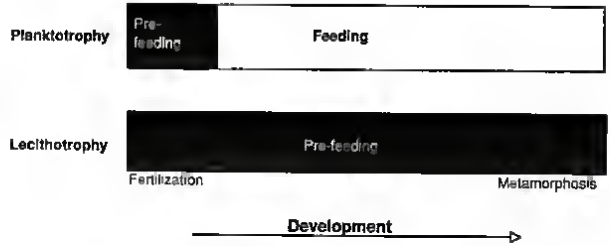


Figure 17 Diagram based on Vance 's model (1973a) comparing the duration of the prefeeding and feeding stages in both lecithotrophic and planktotrophic species during larval development (Herrera *et al* , 1996)

The relationship among development time, larval type, and egg size has been re-examined many times (Vance 1973b, Strathmann, 1977, Christiansen & Fenchel, 1979, Roughgarden, 1989, Havenhand, 1995). All of these models predict that there are two egg sizes which are favored by selection.

Facultative Planktotrophy

A few species with an intermediate strategy have been discovered. They did not fit clearly into the two widely recognized nutritional strategies, planktotrophy (feeding) and lecithotrophy (nonfeeding). The larvae of these species can eat and use particulate food but they do not need to feed to reach metamorphosis. This reproductive strategy has been described as "facultative planktotrophy" (Chia, 1974, Kempf & Hadfield, 1985). These species are either gastropod molluscs. *Adalaria proxima*, (Thompson, 1958, Kempf & Todd, 1989), *Phestilla sibogae* (Kempf & Hadfield 1985), and *Conus pennaceus* (Perron, 1981), or echinoid echinoderms *Clypeaster rosaceus* (Emlet, 1986), and *Brissopsis lyrifera* (Strathmann, 1978a, Hart, 1996) and other spatangoids (heart urchins). Facultative feeding also occurs in anomuran crabs, caridean shrimp (McConaugha, 1985), and at least one species of toad (Crump, 1989), though it has not be described as facultative planktotrophy in these fauna.

Facultative planktotrophy is a mixture of traits from the more typical patterns of larval nutrition. Facultative planktotrophs have been seen as an intermediate larval type. But so few species have been found that have this type of developmental pattern that it appeared a minor component of the ecological diversity of larvae. This assumption was supported by traditional models which predict that species with intermediate levels of egg

energy reserves will have lower "reproductive efficiency" (Vance, 1973a) Intermediate species were expected to be rare

These facultatively planktotrophic larvae are functionally lecithotrophic (Herrera *et al*., 1996, McEdward, 1997), they can reach metamorphosis using only the energy stored in the egg, yet they are able to feed. Feeding lecithotrophic larvae do not fit the traditional definition of planktotrophy or lecithotrophy Thus it is necessary to distinguish between the ability to feed and the requirement for food Kempf and Todd (1989) and Herrera *et al*., (1996) provide definitions which separate and describe these nutritional strategies feeding larvae = larvae that can capture and utilize exogenous food; nonfeeding larvae = larvae that cannot capture or utilize exogenous food; planktotrophic larvae = larvae that require exogenous food for development to metamorphosis; lecithotrophic larvae = larvae that do not require exogenous food for development to metamorphosis.

Egg Size Differences

Many closely related species with feeding larvae often have very different egg sizes (e.g., *Clypeaster subdepressus* [152µm = 1 84µl] and *C. rosaceus* [280µm = 11 49µl] Emlet, 1986; *Strongylocentrotus purpuratus* [85µm = 0 32µl] and *S. droebachiensis* [155µm = 1 95µl] Strathmann & Vedder, 1977) Thus, egg size is a life history characteristic that can easily change Selective pressures which may lead to the increase of egg size in species with feeding larvae probably include selection for decreased pelagic period (Strathmann, 1985), shorter generation time (Havenhand 1993), and higher fertilization success (Levitan, 1993) Selective pressures which might cause an increase in egg size in species with nonfeeding larvae include selection for an increase in juvenile size

(Lawrence *et al.*, 1984, Emlet & Hoegh-Guldberg, 1997) or an increase in post metamorphic energy stores. Because the material necessary to build the juvenile is already present within the egg, pelagic period and generation time are less likely to be affected by an increase in egg size in species with nonfeeding larvae. Thus, some of the selective pressures which act to increase egg size in species with feeding larval development, and possibly lead to a transition from feeding to nonfeeding development, may not be the same pressures which lead to increases in egg size after the transition to nonfeeding larval development (Wray, 1995).

Facultative Feeding Life History Model

The most recent advances in life history modeling recognize the advantages of intermediate egg sizes (McEdward, 1997). The advantages of intermediate egg sizes stem from the ability of these larvae to facultatively feed during the time between the onset of feeding and the need to feed (Herrera *et al*., 1996). More of their larval development is fueled by endogenous reserves than in species with extreme planktotrophy and very small egg sizes. The advantages of these intermediate nutritional strategies include less susceptibility to food limitation, rapid rates of development with most of larval development fueled by egg energy content, lower mortality due to rapid development and metamorphosis (McEdward, 1997). McEdward's model (1997) predicts that intermediate egg sizes will be favored, under a variety of feeding conditions. Food is factored into the model as a percentage of the amount required for the maximal rate of development. The Facultative Feeding Model (McEdward, 1997) predicts a higher reproductive efficiency (% metamorphs) for species with intermediate egg sizes.

The predictions from Vance's model have been assumed to be supported by the bimodal distribution of egg sizes among species in many taxa (e g , echinoids and asteroids, Emlet *et al.*, 1987, but not molluscs, Kohn and Perron, 1994) The apparent dichotomy of egg sizes in some taxa was seen as empirical support of the theory that producing only very large or very small eggs yields the highest reproductive efficiency. McEdward (1997) recognized that the maximum egg size treated by Vance's model (s=1) actually represents facultative planktotrophy (the threshold of lecithotrophy) Nonfeeding larvae from very large eggs fall outside the range treated by traditional life history models Traditional models evaluate only the lower end of the bimodal distribution, and there is a range of nutritional strategies within that distribution.

Intermediate Nutritional Strategies

Recently, a number of species that have larvae with intermediate nutritional patterns have been identified (Eckert, 1995, Herrera *et al* , 1996) These echinoids are from the subtropical Atlantic and Gulf of Mexico. There had been few studies of larval feeding and nutrition on this fauna This study identifies a number of species with intermediate egg sizes and nutritional strategies. These strategies fall between extreme planktotrophy and facultative planktotrophy (functional lecithotrophy), and they are probably the result of different selective pressures not accounted for by traditional life history models McEdward's new model (1997) takes into account the prefeeding and feeding portions of planktotrophic development and recognizing the advantages of a period of facultative feeding predicts that a range of egg sizes may be favored by selection.

The study of subtropical echinoids provides the empirical basis for the facultative feeding model and illuminates the need for continued reevaluation of life history models.

Methods

Eight species of sea urchins were raised following the methods presented in Chapter Two. These species were *Arbacia punctulata* (Lamarck), *Lytechinus variegatus* (Lamarck), *Mellita quinquiesperforata* (Leske), *Clypeaster subdepressus* (Gray), *Encope michelini* L. Agassiz, *Encope aberrans* Martens, *Leodia sexiesperforata* (Leske), and *Clypeaster rosaceus* (Linnaeus). In this experiment, all larvae were raised at 27°C and were either fed 8 cells μl^{-1} *Dunaliella tertiolecta* or were starved. Larvae were tested for metamorphic competence at the first sign of rudiment formation, and every day thereafter, by exposure with 40mM excess KCl for 15 minutes (Cameron *et al*., 1989). After exposure, larvae were observed for metamorphosis periodically for 24 hours.

Clypeaster subdepressus was collected offshore from Cedar Key Florida (28°57'86"N, 83°13'65"W) on September 1, 1992. *Clypeaster rosaceus* was collected offshore from Long Key, Florida on October 14, 1992. *Leodia sexiesperforata*, *Encope michelini*. *Encope aberrans* and *Mellita quinquiesperforata* were collected offshore from Cedar Key, Florida (28°57'30"N, 83°13'02"W) on May 11, 1993. *Lytechinus variegatus* was collected offshore from Cedar Key, Florida (28°59 84"N, 83°12'35"W) on June 7, 1993. *Arbacia punctulata* was collected offshore from Anclote, Florida (28°09'97"N, 83°02'43"W) on April 16, 1994. All species were collected using SCUBA at depths of 5 - 10 meters.

Egg energy content was determined, in collaboration with E Nicu, for the eggs of *A. punctulata*, *L. variegatus*, and *E. aberrans*. Procedures followed those outlined in McEdward and Carson (1987).

Results

Time to the initial feeding stage (4pl) is very short, for all of the species studied (Table 5). Time to the 4pl stage was not correlated with egg size (Spearman $r_s = 0.0774$, $P < 0.05$). Time to the 8pl stage was inversely related to eggsize (Spearman $r_s = 0.6417$, $P < 0.001$). Times for development to metamorphosis range from 3 weeks for *Arbacia punctulata* to 5 days for *C. rosaceus* and are inversely related to egg size (Spearman $r_s = 0.7120$, $P < 0.001$). Juvenile sizes range from 678µm in diameter for *A. punctulata* to 260µm diameter for *L. sexiesperforata* and again, are inversely related to egg size (Spearman $r_s = 0.8058$, $P < 0.001$).

Development without Feeding

Arbacia punctulata had an egg diameter of 76 ± 1µm and an egg energy content of $1.99 \times 10^{-3} \pm 0.6 \times 10^{-3}$ joules (Table 5). *Lytechinus variegatus* had an egg diameter of 107 ± 1µm and an egg energy content of $3.32 \times 10^{-3} \pm 0.8 \times 10^{-3}$ joules (Table 5). The larvae of both *A. punctulata* and *L. variegatus* reached the initial feeding stage (4pl) by the second day (Table 5). Without feeding, these larvae developed only to the 4pl stage. *Mellita quinquesperforata* had an egg diameter of 110 ± 1µm and reached the initial feeding stage (4pl) in less than 1 day (Table 5). *M. quinquesperforata* developed only to the 6pl stage without feeding (Table 5).

Table 5 Degree of dependence on exogenous food among echinoid larvae [a] egg size, energy contents from Emlet, 1986 Juvenile size test diameter (without spines) Culture conditions temperature = 27°C, food = *Dunaliella tertiolecta*, 8 cells μl^{-1}, * = not fed. Time to metamorphosis is the day on which at least 50% of the induced larvae metamorphosed.

Species	Egg Size µm n=30	Eggsize joules n=4-7	Time to 4pl (d) n=1200	Development without Food n=600	Time to 6pl (d) n=600	Time to Metamorphosis with Food (d) n=10	Juvenile Size µm n=7-0
Arbacia punctulata	76±1	1.00×10^{-3} $\pm 0.6 \times 10^{-3}$	2	4-pl	6-8	21	678±3
Lytechinus variegatus	107±1	3.32×10^{-3} $\pm 0.8 \times 10^{-3}$	2	4-pl	6	10	484±4
Mellita quinquesperforata	110±1		<1	4-pl	2.5	7	347±3
Clypeaster subdepressus	150±1		2	4-pl	4	11	295±3
Encope aberrans	189±1	4.01×10^{-3} $\pm 0.9 \times 10^{-3}$	1	8-pl	2.5	7	281±3
Encope michelini	198±1		1	8-pl	2	9	306±3
Leodia sexiesperforata	208±1		<1.5	8-pl	2	7	287±3
Clypeaster rosaceus	284±1	2.06×10^{-2}	2	juvenile	3	5*	290±2*

Several species have egg sizes greater than 150 µm. *Clypeaster subdepressus* had an egg diameter of 150 ± 1µm and reached the initial feeding stage (4pl) by the second day (Table 5). *C. subdepressus* developed to the 8pl stage by day 4 without feeding. *Encope aberrans* had an egg diameter *of* 189 ± 1µm and an egg energy content of $4.01 \times 10^{-3} \pm 0.9 \times 10^{-3}$ joules (Table 5). The larvae of *E. aberrans* reached the initial feeding stage (4pl) by the first day (Table 5) and developed to the 8pl by day 2.5, without feeding. *Encope michelini* had an egg diameter of 198 ± 1µm and reached the initial feeding stage (4pl) by the first day (Table 5). The larvae of *E. michelini* developed to the 8pl by day 2 without feeding. *Leodia sexiesperforata* had an egg diameter *of* 208 ± 1µm and reached the initial feeding stage (4pl) in 1.5 days (Table 5). The larvae of *L. sexiesperforata* developed to the 8pl by day 2 without feeding. None of these species reach metamorphosis without feeding. *Clypeaster rosaceus* had an egg diameter *of* 284 ± 1µm and reached the initial feeding stage (4pl) by the second day (Table 5). The larvae of *C. rosaceus* developed to the 8pl by day 3 and through metamorphosis without feeding. Juvenile size was 290 ± 2 µm (Table 5).

Development with Feeding

With feeding, the larvae of each species reached the initial feeding stage (4pl) at the same time as did their sibling larvae in starved treatments. With feeding, the larvae of *A. punctulata* reached the 8pl stage by day 6-8 and metamorphic competency was reached at day 20-21. Juvenile size was 678 ± 3µm. The larvae of *L. variegatus* were 8pl by day 6, metamorphic competency was reached at day 10-12, and juvenile size was 484 ± 4µm.

The larvae of *M. quinquesperforata* were 8pl by day 2.5, metamorphic competency was reached at day 5-7, and juvenile size was 347 ± 3µm (Table 5)

Among species with egg diameters of 150 µm, or more, with feeding, *C. subdepressus* reached metamorphic competency at day 11 and juvenile size was 295 ± 3µm (Table 5). *E. aberrans* reached metamorphic competency at day 5-7 and juvenile size was 281 ± 3µm. *E. michelini* reached metamorphic competency at day 9 and juvenile size was 306 ± 3µm (Table 5) *L. sexlesperforata* reached metamorphic competency at day 6-7 and juvenile size was 258 ± 3µm (Table 5)

Discussion

These studies on subtropical echinoids (mostly clypeasteroids) have revealed a diversity of energetic strategies, and a range of egg diameters (Table 5). The egg diameters for these species range from 76 to 284µm. The eggs of *Clypeaster rosaceus* (284µm diameter) contain ~10 times more energy than the eggs of *Arbacia punctulata* (76µm diameter) and *Lytechinus variegatus* (107µm diameter) In those species previously studied, egg sizes are similar to or the same as the egg sizes reported here (Harvey, 1956, Mazur & Miller, 1971, Caldwell, 1972, Emlet, 1986, Eckert, 1995, Lessios, pers. comm. as cited in Emlet *et al* , 1987, and Emlet, pers. comm.), except for *Leodia sexlesperforata* (reported egg sizes ranging from 164µm [Emlet, pers. comm.] to 260µm [Crozier, 1918 as cited in Emlet *et al.*, 1987] in diameter) Crozier's measurements may have included the egg jelly coat

Time to the initial feeding stage (4pl) is very short, only 1 to 2 days, for all of the species studied (Table 5) and was not correlated with egg size Time to the fully

developed 8pl stage is very short in most of the clypeasteroids (*Mellita, Encope Clypeaster rosaceus*, and *Leodia*), with egg sizes from 110µm to 284µm. However, for the regular urchins (*Arbacia* and *Lytechinus*) with egg sizes of 76 and 107µm, the time to the fully developed 8pl is longer (Table 5) Time to the 8pl stage was inversely related to eggsize

As egg size increases, time to metamorphosis and juvenile size decrease (Table 5) Times for development to metamorphosis range from 3 weeks for *Arbacia punctulata* to 5 days for *C. rosaceus* and are inversely related to egg size. Juvenile sizes range from 678µm in diameter for *A punctulata* to 260µm diameter for *L. sexiesperforata* and again, are inversely related to egg size (Table 5). The time to metamorphosis and juvenile size data from previous studies by other authors often differs substantially from mine (*Arbacia* [Harvey, 1956], *Lytechinus* [Mazur & Miller, 1971], *Clypeaster subdepressus* [Emlet, 1986]) These previous studies were carried out under various temperature and culturing regimes, used different species and combinations of food organisms, and probably lacked a common criterion for timing the induction of metamorphosis. A common set of temperature, feeding, and culturing conditions (Table 5), in combination with consistent criteria and methods for the induction of metamorphosis, allows a more reliable comparison of growth and development in these eight species of echinoids. Previous studies in which the methods were consistent with ours obtained similar time to metamorphosis and/or juvenile size data for some species (*Mellita quinquesperforata* [Caldwell, 1972], *Encope michelini* [Eckert, 1995], and *Clypeaster rosaceus* [Emlet, 1986])

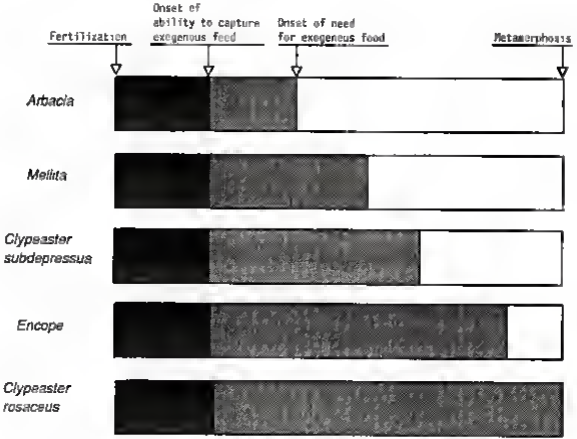
Larvae of species with larger eggs (≥150 µm diameter) (*Clypeaster subdepressus*, *C. rosaceus*, *Encope michelini*, *E. aberrans*, and *Leodia sexiesperforata*) are less dependent on exogenous food. In these species, later stages of larval development can be reached without feeding (Table 5). In species with smaller eggs (≤110µm diameter) the developmental stage which can be reached without exogenous feeding ranges from the 4pl stage for *Arbacia punctulata* and *Lytechinus variegatus*, to the 6pl stage for *Mellita quinquiesperforata*. In subtropical clypeasteroid larvae with egg sizes at or exceeding 150µm in diameter (e.g., *L. sexiesperforata*, *C. subdepressus*) the 8pl stage is reached, without feeding. In *Clypeaster rosaceus*, with an egg diameter of 284µm, the juvenile rudiment is formed without feeding. All of these echinoid species, except *C. rosaceus*, require food to build the juvenile rudiment and metamorphose.

The development of species with feeding larvae has been divided into two sequential stages: prefeeding and feeding (Vance, 1973a; Strathmann, 1985; Havenhand, 1995). The division between the prefeeding and feeding stages is marked by the onset of larval feeding activity (Fig. 17). Echinoids with planktotrophic development have a relatively short prefeeding period during which they are obligately lecithotrophic and utilize endogenous nutrient reserves from the egg to build the initial larval stage. The construction of larval feeding structures, such as ciliated bands and a digestive system, provides the offspring with the ability to process the surrounding seawater and eat suspended food particles. Lack of food results prevents further development (addition of larval arms) and growth (increase in size or biomass) and eventually leads to deterioration and death.

No echinoid species is known in which the larva has to feed as soon as it is able to feed. However, many species do need to feed within a day or two of developing the ability to feed. Relative to the total feeding period, from the initial feeding stage to metamorphosis, the ability to capture food is nearly coincident with the need to utilize food in most echinoids (Fenaux *et al*., 1988, McEdward & Herrera, in press). In contrast, there is a diversity of nutritional strategies among subtropical echinoids. In this fauna there is a dissociation of the two aspects that comprise the "onset of feeding" (Fig. 18) (Herrera *et al*., 1996). Among species with feeding development, the larvae are able to feed by the early 4pl stage. But there is a range of stages at which larvae become dependent on exogenous food (Fig. 18), and the degree of dependence is correlated with the amount of endogenous reserves in the egg (Table 5) (Herrera *et al*., 1996).

Arbacia punctulata is an extreme obligate planktotroph, which does not develop beyond the 4pl stage without feeding. *Mellita quinquiesperforata* can develop to the 6pl larval stage before it needs to feed (McEdward & George, in prep., Chapter 5). However, development cannot proceed beyond the 4pl stage in this species, if the amount of endogenous reserves is experimentally reduced by half (Chapter 5). *Clypeaster subdepressus*, *Leodia sexiesperforata*, *Encope michelini* and *Encope aberrans* all develop to the 8pl larval stage without feeding, even though they are able to feed at the 4pl stage (Fig. 18, Table 5). All of these species are obligate planktotrophs and must feed to reach metamorphosis, but they differ in the degree of dependence on exogenous feeding. *Encope michelini* has very limited dependence on feeding and can complete development through metamorphosis with only 3 days of feeding at any time during larval development (Eckert, 1995). *Clypeaster rosaceus* can feed at the 4pl larval stage, but can complete

Figure 18 From the study of subtropical echinoderm species, it now appears that the feeding stage includes two critical points in time: the onset of the capacity to feed and the onset of the need to feed. The onset of the ability to feed occurs at the same stage of development but the onset of the need to feed varies dramatically from species to species (Herrera *et al.*, 1996)


Fertilization
Onset of ability to capture exogenous feed
Onset of need for exogeneus food
Metamorphosis
Arbacia
Melita
Clypeaster subdepressus
Encope
Clypeaster rosaceus

larval development through metamorphosis without feeding. *C. rosaceus* is able to feed at the same stage as all other planktotrophic echinoid larvae, but never needs to feed (Fig 18). It is the only species in this study that fits the definition of "facultative planktotrophy". *C. rosaceus* represents the extreme dissociation of the ability to feed and the need to feed and has a functionally lecithotrophic pattern of development. This range of nutritional strategies reveals that lecithotrophy and planktotrophy are ends of a continuum of energetic strategies and should not be considered as fundamentally different nutritional patterns (Herrera *et al*., 1996, McEdward & James, 1997, McEdward, 1997).

In *Lytechinus variegatus*, a species with a small egg diameter (110µm), higher levels of exogenous food allow the larvae to reach metamorphosis sooner than do sibling larvae fed lower levels of food. However, juvenile size does not appear to be affected (Chapter 2). In *Clypeaster rosaceus*, a species with a larger egg (284µm), feeding allows the building of a larger juvenile but does not cause a decrease in the time to metamorphosis (Emlet, 1986). There may be a minimum amount of time necessary to build the juvenile rudiment, and thus in species near the threshold to lecithotrophy, with short development times, excess external food does not shorten development time further, but rather is utilized to increase the size of the rudiment. In nonfeeding species, as egg size increases, development time is not shortened (McEdward, 1997) by the presence of excess endogenous resources, rather the resultant juvenile is larger (Emlet *et al*., 1987), and presumed to be of higher quality (McEdward 1997). These differences in growth and development suggest that there are different responses and tradeoffs among species employing different energetic strategies. Larval nutritional strategies depend on the level of maternal investment available within the egg. Extreme planktotrophs gain the

advantages of increased fecundity, obligate planktotrophs with larger egg sizes are much less dependent on early feeding, some species have very rapid development times, and it has been hypothesized that they have the advantage of an increased ability to delay metamorphosis (Havenhand, 1995). Facultative planktotrophs (functional lecithotrophs) have the advantages of insusceptibility to starvation, very rapid development times, and increased juvenile sizes with feeding. Nonfeeding lecithotrophic larvae have rapid development times and can utilize excess endogenous reserves to produce large juveniles without feeding.

Several studies have been conducted manipulating (via blastomere separations) the level of endogenous food available to the developing larvae, and these studies reveal that reduced egg size can change the stage of larval development that can be attained with endogenous reserves alone (Chapters 5 and 6; Herrera, 1995; McWeeney, Herrera, & McEdward, in prep.). This indicates a direct link between the level of parental investment and the degree of dependence on exogenous food.

Species with intermediate energetic strategies are thought to represent evolutionary transitions between planktotrophy and lecithotrophy (Emlet, 1986; Eckert, 1995; Herrera *et al.*, 1996). As egg size increases, a functional threshold to lecithotrophy may be crossed (Fig. 1B) (Herrera *et al.*, 1996). These larvae are lecithotrophic, but they retain feeding structure and capability. The morphological differences observed between feeding and nonfeeding larvae would require changes in morphogenesis (McEdward & Janies, 1997), and once complex larval feeding structures are lost, they probably cannot be re-evolved (Strathmann, 1978b; McEdward & Janies, 1997).

There is greater diversity of nutritional strategies than has been previously recognized. Differences in egg energy contents (maternal investment) determine how much external food is required to complete larval development and metamorphose. There is a continuum of nutritional strategies between extreme obligate planktotrophy and functional lecithotrophy (facultative planktotrophy) (Herrera *et al.*, 1996, McEdward & Janies, 1997). This study supports the hypothesis that the ecological change between planktotrophy and lecithotrophy might be easily accomplished by a relatively slight increase in egg size, and that unlike the extensive morphological changes usually associated with the transition from feeding to nonfeeding development, the ecological transition from planktotrophy to lecithotrophy might be easily reversed (Herrera *et al.*, 1996, McEdward & James, 1997).

In contrast to traditional interpretations and predictions from life history theory, facultative planktotrophy and other intermediate nutritional patterns in larvae could potentially be important ecological strategies. This study provides the first empirical basis for recent advances in life history theory (McEdward, 1997). The diversity of nutritional strategies, identified in the species in this study, is probably not limited to the echinoid echinoderms. Further studies in a range of taxa, recognizing intermediate strategies in species with relatively small eggs (in comparison to species with nonfeeding development), are needed.

CHAPTER 5
THE EFFECT OF AN EXPERIMENTAL CHANGE IN EGG SIZE ON LARVAE OF THE SAND DOLLAR *MELLITA QUINQUIESPERFORATA*

Introduction

Egg size is a central trait in the ecology and evolution of marine invertebrate life histories (Sinervo and McEdward, 1988). In the past, many fecundity/time models of marine invertebrate life histories have been suggested as explanations for the different life history strategies observed in these animals (Vance, 1973a, b; Christiansen and Fenchel, 1979; Strathmann, 1985, Roughgarden, 1989, McEdward, 1997). The main assumption of these models is that the amount of energy that the parent invests per offspring will determine the fitness of those offspring. Echinoids free spawn and do not provide any parental care to their young, thus the endogenous resources packaged in the egg represent the entire parental investment. Many life history models predict that eggs which hatch as planktotrophic larvae will be small (Vance, 1973a, b, Christiansen and Fenchel 1979, Strathmann, 1985, Roughgarden, 1989). This allows for the highest level of fecundity possible by providing only enough energy in each egg for the development of the early feeding larval stage, yielding larvae that are obligately planktotrophic very early in life. In fact, many planktotrophic larvae do hatch from relatively small eggs (50 – 190μm diameter, Emlet *et al.*, 1987).

Among echinoids, with planktotrophic larvae, there is a range of egg sizes, a range of endogenous reserves (0.001-0.02 joules, Herrera *et al.*, 1996, Emlet 1986),

and a diversity of larval nutritional strategies (Herrera *et al*., 1996, Chapter 4). Some of these larvae can develop to later larval stages (6pl, 8pl) without feeding (Eckert, 1995; McWeeney, 1995; Herrera *et al*., 1996, McEdward & George, in prep.). These species have egg sizes that are larger than required for building the initial feeding larval form.

Endogenous resources in the egg can be manipulated with experimental embryological techniques. Blastomeres can be isolated at the 2-cell stage yielding half-size eggs. These "half-size eggs" will develop into normal larvae which are capable of complete development through metamorphosis when larvae are provided with sufficient exogenous food (Horstadius, 1939, 1973, 1975, Harvey 1940, Okazaki and Dan, 1954, Hinegardner, 1975; Marcus, 1979, Sinervo and McEdward, 1988, Hart, 1996, Chapter 6).

In many echinoid species there is more nutritional material in the egg than traditional life history models would predict (Emlet *et al*., 1987, Herrera *et al*., 1996). *Mellita quinquiesperforata* was chosen for this study because it has an egg-size (110µm diameter) which is intermediate within the range of egg-sizes (50-190µm diameter, Emlet *et al*. 1987) among species with planktotrophic development, and because its egg provides more than enough energy to develop beyond the initial feeding larval stage (4pl). The larvae of *M. quinquesperforata* can develop to the 6pl stage without feeding (McEdward & George, in prep.). Can larvae from eggs with half the usual endogenous reserves develop to the same larval stage as their siblings from full-sized eggs? Larvae of echinoids with the same size (*Lytechinus variegatus*) and smaller (*Arbacia punctulata*) eggs than *M. quinquiesperforata* can reach only the 4pl stage on endogenous reserves alone (Herrera *et al*., 1996, Chapter 4). Thus, it was hypothesized that *M. quinquiesperforata* larvae from half-size eggs would not be able to reach the 6pl stage

without an exogenous source of particulate nutrition, and that they might only be able to reach the 4pl stage before they began to deteriorate and die.

The ability to attain a later stage of development using endogenous resources alone is an important adaptation because feeding structures are more extensive in later stages (McEdward 1986a, b). Growing more arms increases the length of the ciliated band, increasing the clearance rate of food particles, and this would increase feeding success when resources are patchy or food concentrations are low (Strathmann, *et al.*, 1972).

Another means of increasing the length of the larval feeding structure is to increase the lengths of the arms. Larvae with longer arms do have higher clearance rates compared to larvae with shorter arms (Hart & Strathmann, 1994). Echinoid larvae are capable of increasing the lengths of their arms in response to low food concentrations (Boidron Metairon, 1988, Hart & Scheibling, 1988, Strathmann *et al.*, 1992, Fenaux *et al.*, 1994). This increase in arm length is a form of phenotypic plasticity.

The larvae of *M. quinquiesperforata* have also been documented as exhibiting phenotypic plasticity (McEdward & George, in prep.). A reduced egg-size might not only change the stage of development larvae can reach without feeding, it might also affect the ability of the larvae to grow longer arms in order to compensate for limited food concentrations early in development. Larvae from reduced eggs (88µm diameter, calculated) are not expected to be able to express phenotypic plasticity early in development when larval growth is supported by endogenous reserves.

This study is an examination of the effect of an experimentally induced intraspecific difference in endogenous reserves on development in echinoid larvae. As a

result of reducing the amount of growth and development that larvae can accomplish on maternal reserves, the facultative feeding period will be reduced, and the ability of the larvae to efficiently acquire exogenous resources will be more critical to successful growth and development. A reduction in endogenous reserves might cause a decrease in the ability of larvae to adjust morphology to compensate for reduced food concentrations and might extend the amount of time larvae will need to feed in order to reach metamorphosis. Any of these effects: a reduction in the amount of growth and development supported by endogenous sources, a reduction in the facultative feeding period, or a decrease in the larvae's ability to express phenotypic plasticity will require that the larvae spend a longer period feeding in the plankton and will reduce developmental success.

This study was done in collaboration with S.K. McWeeney. The effects of an experimental manipulation of egg size in *Mellita quinquiesperforata* on those larvae fed unlimited food or starved is the focus of this chapter. A more extensive review of the effects of unlimited vs. limited food is presented in McWeeney (1995).

Methods

Adults of the sand dollar *Mellita quinquiesperforata* were collected from Sea Horse Key, Florida (29°06.15'N, 83°03.60'W) in June, 1994. Adults were maintained and spawned as outlined in Chapter Two.

Blastomeres were isolated using the procedures of Okazaki (1975). The eggs were fertilized (100%) and rinsed in calcium-magnesium free seawater (CaMgFSW, for formula see Strathmann, M.F., 1987). As soon as the fertilization envelope was evident,

it was stripped from around the eggs by passing them through a 70μm Nitex mesh. Eggs were placed in agar-coated dishes in calcium-free seawater (CaFSW, Strathmann, M.F., 1987). Full size controls were removed from the CaFSW treatment at the beginning of the first cell division and placed in agar-coated dishes in normal seawater with 0.3g streptomycin liter^{-1} to prevent infection.

In the embryos remaining in the CaFSW, blastomeres were separated at the two cell stage by passing them through 64μm Nitex mesh two times. After the blastomeres were isolated, they were placed in agar-coated culture dishes in normal seawater with 0.3g of streptomycin liter^{-1} and all cultures were placed in the culture chamber and maintained at a temperature of 27°C. At the blastula stage, embryos were cultured as outlined in Chepter Three. All culture water was filtered (0.45μm) and 0.3g streptomycin liter^{-1} added.

Three nutritional treatments were done for both full and half-sized embryos and each treatment was duplicated. Based on the results of the nutrition experiments described in Chapter Two, the treatments chosen for this experiment were: 8 cells μl^{-1} (unlimited food), 2 cells μl^{-1} (limited food), and 0 cells μl^{-1} (starved) of the green alga *Dunaliella tertiolecta* (Butcher). Survival, development rates, and time to metamorphosis of the larvae in each culture were observed and recorded.

Morphological measurements were made on the larvae cultured under unlimited and limited food levels. Larvae from the unlimited food and limited food treatments were killed with 1% formalin in seawater and stored in 70% ethyl alcohol (EtOH). Twenty larvae were collected every 12 hours after fertilization for the first three days, and then every 24 hours until the end of the experiment. Morphometric measurements

were made on the skeletons of the preserved larvae using the methods of McEdward & Herrera (in press). The results of these measurements are reported in McWeeney (1995).

To compare stages across treatments, equivalent stages were determined for larvae from full and half-size eggs, fed limited or unlimited food, by shape-fitting statistics (see McWeeney, 1995). The shape of each larva was compared with the shapes of all of the other larvae. Staging was judged using only larval features, no juvenile structures were measured.

Results

Early Development

Early development of the larvae from half-size eggs (blastomeres from the 2-cell stage) was delayed in comparison to that of the controls (from full-size eggs) (Figure 19). At 0.5 day of age, embryos from the separated blastomeres were mesenchymal blastulae, while those from whole eggs had completed development through the gastrula, begun larval skeleton formation, and were at the prism stage. At one day of age, larvae from half-size eggs were at the 2pl stage and larvae from the control group were at the 4pl stage.

Later Development in Starved Larvae

Starved larvae from the half-size eggs reached only the 4pl stage, and starved larvae from the full size eggs reached the 6pl stage before they began to deteriorate and die. In starved larvae from half-size eggs, the larval body was thin and the skeletal rods in the arms began to protrude from the tips of the arms during the 4pl stage. There was

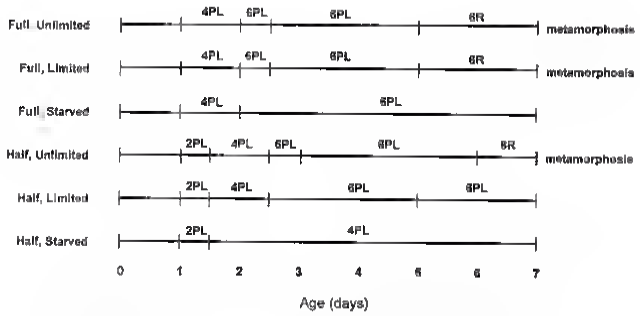


Figure 19 Schedule of larval development and metamorphosis in *Melitta quinquiesperforata* Treatments = full-size and half size eggs, fed unlimited or limited amounts of food or starved.

no evidence of formation of the dorsal arch or posterodorsal skeletal rods, or of formation of the third pair of arms, the posterodorsals. In starved larvae from full-size eggs there was no evidence of formation of the fourth pair of arms, the preorals. In the starved cultures, of both egg-size treatments, there was high mortality throughout the later part of the experiment (after the 4pl stage was formed in the half-size treatments and after the 6pl stage was formed in the full-size treatments).

Later Development in Fed Larvae

In the fed treatments, as development continued, it was noted that larvae from the half-size eggs were approximately 12 hours behind the controls in forming each larval stage. Fed larvae from full-size eggs, attained the 6pl stage at 48 hours, the 8pl stage at 60 hours, and the 8R stage at 120 hours after fertilization, regardless of food level. Larvae from half-size eggs were at the 6pl stage at 60 hours. Larvae from half-size eggs fed unlimited food reached the 8pl stage at 72 hours and the 8R stage at 144 hours. Larvae from half-size eggs fed limited food reached the 8pl stage at 120 hours and did not grow a rudiment during the course of the experiment. The larvae from full-size eggs on limited or unlimited food built the juvenile rudiment earlier than the larvae from half-size-eggs fed unlimited food.

Effect of a Reduction in Egg Energy Content on the Expression of Phenotypic Plasticity

Larval arm length among treatments during early development (day 1)

Larvae from full-size eggs fed limited food had longer postoral arms than larvae from full-size eggs fed unlimited food less than 1 day postfertilization (Table 6).

(McWeeney, 1995) In the larvae from half-size eggs, there was no difference in arm length in the early stages (McWeeney, 1995)

Table 6 Skeletal arm rod length measurements for blastomere separation cultures Mean ± SE in µm with N=10 All from same parental pair (from McWeeney, 1995)

Measurement	Treatment (egg-size)	Food level (cells μl^{-1})	Age (days)	Mean length (µm)
Postoral arms				
	Full	8	1	209 95 ± 5 4
	Full	2	1	228 75 ± 5 5
	Full	8	3	432 ± 6 3
	Full	2	3	458 ± 8 4
	Half	8	3	332 ± 12 2
	Half	2	4	372 ± 12 8
	Half	8	4	312.5 ± 8 9
Posterodorsal arms				
	Full	8	3	211 ± 10.5
	Full	2	3	274 ± 25 1

Larval arm length among treatments during later development (days 3 and 4)

In larvae from full-size eggs those fed limited food had longer postoral and posterodorsal arms than those fed unlimited food at the same stage (Table 6) (McWeeney, 1995) Larvae from half-size eggs fed limited food had longer postoral arms at the 6pl stage than did larvae from half-size eggs fed unlimited food, at the

equivalent stage (Table 6) (McWeeney, 1995). The postoral arms were also longer in larvae from half-size eggs fed limited food in comparison to those of the larvae from half-size eggs fed unlimited food which were a stage ahead of them (8pl stage) (Table 6) (McWeeney, 1995). There were no differences in the lengths of the anterolaterals at the 6pl stage (McWeeney, 1995). Measurement of later larval stages was not possible due to splaying of the later stage larvae. In splaying, the larval arms are spread wide and this prevents the positioning of the larvae for morphometric measurements.

Discussion

Effect of a Reduction in Egg Energy Content on Development

Early development (fertilization - early 4pl)

The effect of a reduction in egg energy content was most apparent during the early stages of development, before the formation of the first pair of larval arms. Early development of the larvae from half-size eggs (blastomeres from the 2-cell stage) was delayed in comparison to that of the larvae from full-size eggs.

Later larval development in starved larvae (late 4pl - metamorphosis)

In starved larvae, a reduction of egg energy content by 50 percent prevented the larvae from developing to the 6pl stage. In larvae from full-size eggs, there was no evidence of formation of the fourth pair of arms, the preorals. Larvae of *M. quinquiesperforata* from full-size eggs were not able to reach the 8pl stage or metamorphose without feeding. The energy in the egg is insufficient to support further development in either of these starved treatments. The ability of larvae to attain a

particular stage of larval development without food depends on the material provided in the egg (see also Table 5, Chapter 4)

Later larval development in fed larvae

Fed larvae from full-size eggs, fed limited or unlimited food, attained each of the later stages (6pl, 8pl, and 8R) 12 hours before the larvae from half-size eggs that were fed unlimited food. Larvae from half-size eggs fed limited food reached the 8pl stage much later (120 hours) and did not grow a juvenile rudiment during the course of the experiment

Formation of the juvenile rudiment

Larvae from full-size eggs fed unlimited food built the juvenile rudiment earlier than the larvae from half size-eggs fed unlimited food. This suggests that the exogenous energy acquired from the food is allocated differently between the two egg-size treatments. The larvae from full-size eggs are using these resources to build the rudiment at an earlier time. The larvae from half-size eggs are using these resources to continue development of the larval body in compensation for the reduction in egg energy contents.

Effect of a Reduction in Egg Energy Content on the Expression of Phenotypic Plasticity

Plasticity at early stages in development (2pl - 4pl)

Larvae from full-size eggs fed limited food expressed phenotypic plasticity less than 1 day postfertilization. These larvae had longer postoral arms than larvae from full size eggs fed unlimited food (McWeeney, 1995). In the larvae from half-size eggs, there was no difference in arm length between treatments in the early stages (McWeeney,

1995) A reduction in endogenous reserves prevented the expression of phenotypic plasticity early in development.

Plasticity at later stages in development (6pl - 8pl)

Larvae from full-size eggs fed limited food had longer postoral and posterodorsal arms than larvae fed unlimited food at the same stage (McWeeney, 1995) Larvae from half-size eggs fed limited food had longer postoral arms at the 6pl stage than did larvae from half-size eggs fed unlimited food, at either the 6pl or 8pl stages (McWeeney, 1995) At later stages of development, and after some time spent feeding, even larvae from half-size eggs exhibit phenotypic plasticity in response to limited food concentrations. The ability to exhibit phenotypic plasticity at later stages of development is not due to endogenous resources, rather these later stage larvae from half-size eggs can allocate exogenous nutrition to the building of longer feeding structures

General Conclusions

The experimental manipulation of endogenous reserves provides the basis for an intraspecific comparison of larval development given different size eggs. These results suggest new questions about the role of maternal reserves in the expression of life history traits. *M quinquiesperforata* larvae had enough endogenous energy to reach the 6pl stage without feeding. When egg size was halved, starved larvae were no longer able to develop to the 6pl stage and fed larvae took longer to reach metamorphosis

The larvae from full-size eggs fed limited food exhibited phenotypic plasticity early in development, at the early 4pl stage and again, during later development at the 8pl stage Larvae from half size eggs fed limited food expressed phenotypic plasticity

only later in development. A reduction in endogenous resources prevented the larvae from expressing phenotypic plasticity early in development. However, they were able express plasticity later in development, after some time spent gathering exogenous resources via particulate feeding. Increased feeding ability minimizes the effects of limited food conditions on growth and development. The ability to grow longer arms in response to low food levels is derived from endogenous sources early in development, and from exogenous food later in development.

Larvae from relatively large eggs (150-200μm) are able to develop to the 8pl stage without food (Herrera, *et al.*, 1996) and do not appear to grow longer arms in response to low food conditions. Larvae from larger eggs are able to use endogenous energy sources to reach later stages of development. This increases the length of the ciliated band which increases feeding ability and minimizes the possibility that larvae will starve or spend a longer period of time in the plankton gathering the exogenous resources necessary to complete larval development. In species with larger egg sizes, the ability to develop without food to the later stages, may be more beneficial than growing longer arms in response to low food. Developing rapidly to the 8pl stage could allow larvae to compensate for low food conditions by increasing the length of the ciliated band compared to larvae at earlier stages of development. This response would have the same effect as allocating energy to longer arms at an earlier stage. Both this "flexibility" (sensu McEdward & Hadfield, 1996) and phenotypic plasticity allow larvae to compensate for low food conditions, but the current study reveals that the ability to reach a later stage (6pl) without food and/or at an earlier age is dependent upon egg energy content.

CHAPTER 6
THE EFFECT OF AN EXPERIMENTAL CHANGE IN EGG SIZE ON LARVAE OF THE SAND DOLLAR *ENCOPE ABERRANS*

Introduction

Among the echinoids, many species (e g *Paracentrotus lividus*, Fenaux et al., 1988, *Arbacia punctulata* and *Lytechinus variegatus*, Herrera *et al*., 1996) need to feed within a day or at most a few days of developing to the initial larval feeding stage (2 or 4pl) Recently, the larvae of several subtropical species of clypeasteroids have been documented as reaching the 6pl and 8pl stages without food (Eckert, 1995, Herrera *et al*., 1996 McEdward & George, in prep). Although there is not enough material in the eggs of any obligate planktotrophs (by definition) to reach metamorphosis, there appears to be enough variation in reserves among species to generate a diversity of nutritional strategies in development (Herrera *et al* 1996) Intermediate types of planktotrophs are more prevalent than previously thought (Eckert, 1995, Herrera *et al* , 1996)

In many species of subtropical clypeasteroids (sand dollars and sea biscuits), the development of the larval body is fueled by reserves in the egg while the building of the juvenile -- an energetically expensive process (McEdward, 1984) – is fueled by external feeding (Eckert, 1995, Herrera *et al* , 1996) In these clypeasteroids, the complete development of the larval body is very rapid (usually 1-3 days) and overall time to metamorphosis is relatively short (5-7 days) (Herrera *et al*., 1996) These larvae can take

advantage of food in the plankton for building the rudiment and are able to do so early because the larvae develop rapidly using egg energy. Fecundity is still high because the energy contained in these intermediate size eggs is only about 4 times greater than that in smaller echinoderm eggs (Herrera *et al* , 1996, see Chapter 4), while the energy in the eggs of some lecithotrophs is 1-3 orders of magnitude greater (McEdward, 1991; see Chapter 4)

By attaining later larval stages before needing to feed, the larvae are able to spend a period of time feeding facultatively (Eckert, 1995, Herrera *et al* , 1996, McEdward, 1997, Chapter 4). The advantages conferred by this period of facultative feeding have been analyzed in new life history models by McEdward (1997, in prep), and these models predict that intermediate egg sizes and larval nutritional strategies should be favored by selection under a range of environmental conditions.

The subtropical sand dollar *Mellita quinquiesperforata* has an egg size of 110μm (diameter) and its larvae can reach the 6pl stage without feeding. However, larvae of this species can only reach the 4pl stage when endogenous reserves are decreased by half by means of blastomere separations (Chapter 5). In the stronglocentrotid sea urchins it is known that when maternal reserves provided to the offspring are reduced by half, their larval development mimics that of larvae from species with smaller egg sizes (Sinervo & McEdward, 1998)

Larvae of the subtropical sand dollar *Encope aberrans* develop from a larger egg (190μm diameter) than *M. quinquiesperforata*, and *E. aberrans* larvae can reach the final larval stage (8pl), without feeding, although they do require food to develop the juvenile rudiment and metamorphose (Herrera *et al* , 1996). Larvae of the sea biscuit *Clypeaster*

rosaceus (egg size = 284µm diameter) are facultative planktotrophs and can reach metamorphosis without feeding although they are able to feed (Emlet, 1986; Herrera *et al.*, 1996)

In order to investigate the effects of a decrease in egg size in a planktotrophic species with a relatively large egg, and a developmental type intermediate between that of *M. quinquesperforata* and facultative planktotrophy, blastomere separation experiments were done with *E. aberrans*. Larvae of the sea biscuit *Clypeaster subdepressus*, with eggs the same size (150µm diameter) as half-size *E. aberrans* eggs, can reach the 8pl stage on endogenous reserves alone (Herrera *et al.*, 1996, Chapter 4). Thus, it was hypothesized that *E. aberrans* larvae from half-size eggs would be able to reach the 8pl stage without feeding.

If these larvae from isolated blastomeres can develop to the 8pl stage without feeding, then this species packages at least twice the amount of energy in the egg than is necessary for the maximum rate of development (lecithotrophic development) of the larval body. If this is the case, then this species might be approaching the threshold for functional lecithotrophy. This would also indicate that twice the amount of energy needed to develop to the final larval feeding stage is not enough for development through metamorphosis, and metamorphosis is more energetically expensive than growth and development through all the stages of the larval body. If the larvae from isolated blastomeres cannot reach the 8pl stage without feeding, then the energy that *E. aberrans* packages into the egg is less than two times that needed to fuel development of the 8pl larval body. If these larvae reach the 6pl stage, then it is likely that *E. aberrans* packages twice as much energy into the egg as does *M. quinquesperforata*. If the larvae of *E*

aberrans reach only the 4pl stage, then this species puts less than twice as much energy as *M. quinquiesperforata.*

Methods

Adults of the sand dollar *Encope aberrans* were collected offshore from Sea Horse Key, Florida (28°57'30"N, 83°13'02"W) in May, 1994. Maintenance of the adults and spawning, were done as outlined in Chapter Two. The eggs were fertilized and rinsed in calcium-magnesium free seawater (CaMgFSW). Blastomere separation procedures were accomplished as outlined in Chapter Six. In this experiment the fertilization envelope was stripped from around the eggs by passing them through a 130µm Nitex mesh. The embryos for the half-size treatments were separated at the two-cell stage by passing them through 125µm Nitex mesh two times. At the blastula stage larvae were cultured as outlined in Chapter Three. All culture water was filtered (0.45µm) and 0.3g streptomycin liter^{-1} added. Two nutritional treatments were done for both full and half-sized embryos and each treatment was done in duplicate. The nutritional treatments were: 8 cells µl^{-1} (unlimited food) and 0 cells µl^{-1} (starved) of the green alga *Dunaliella tertiolecta* (Butcher).

In each culture, survival, development rates, time to metamorphosis, and juvenile size was observed and recorded. Morphological measurements were made on larvae from each treatment as outlined in Chapter Three. Measurements were done every 12 to 24 hours beginning with the 4pl stage. Statistical analyses and abbreviations are as given in Chapter Three.

Results

General Larval Development of *Encope aberrans*

The fed larvae of *Encope aberrans* from full-size eggs developed through metamorphosis within 7 days at a temperature of 27°C (Table 7, Fig. 20). Larval feeding

Table 7. Schedule of larval development in *Encope aberrans*

Hour of Age (Day)	Stage	Description of Larval Development
24 (1)	2pl	2-arm pluteus. PO arms just extend past the top of oral hood. Evidence of buds of ALA arms. Ventral transverse band simple. Oral hood prominent anterior extension on dorsal side.
36 (1.5)	4pl	PO arms elongating. ALA's longer in most larvae. Evidence of buds of PD's. No elaboration of transverse bands or arm pits. Still a very simple appearing larva.
48 (2)	6pl	Well-developed 6-arm larva. PD arms approximately half the length of PO arms. Buds of PR arms are present. ALA arms well developed. No elaborate arm pits or epaulettes. Slight elaboration of transverse ciliated band between PO arms. Body much simpler than in *Lytechinus variegatus*, lacking many of the lobes and the epaulettes of that species.
60 (2.5)	8pl	PD arms continue to lengthen. ALA arm lengths are somewhat variable. PR arms are variable also, as are POs. PDs are about 1/2 the length of POs in most larvae. Some elaboration of ventral transverse band.
72 (3)	8pl	All arms well-developed and long. PD as long as PO and PR as long as ALA in most larvae. No apparent rudiment formation. Transverse band is further elaborated.
84 (3.5)	8R	Larvae are well-developed, and all arms are long. Rudiment formation is visible on the left side.
96 (4)	8R	Larvae are very large and well-developed. PD is as long as PO in most, and PR is as long as ALA in most. Transverse band is further elaborated and armpit (PO-PD) is deepening.
120 (5)	8R	Arms continue to elongate. Transverse band further elaborated.
144 (6)	8R	Body form has not changed. Arms appear longer.
168 (7)	8R	Arms longer. Tube feet are visible on left side. Onset of metamorphosis.

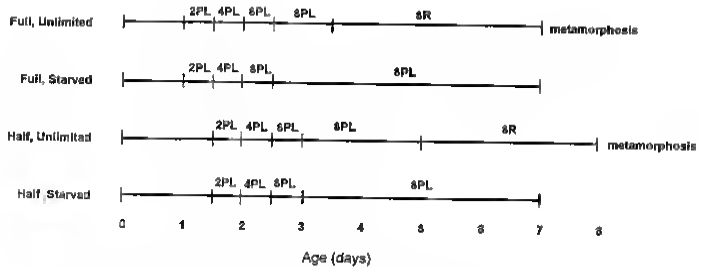


Figure 20 Schedule of larval development and metamorphosis in *Encope aberrans*. Treatments = full-size and half-size eggs, fed unlimited amounts of food or starved.

began by 36 hours (4pl) as evidenced by the presence of algae in the larval gut. The larvae reached the 6pl stage by 48 hours and were 8pl larvae by 60 hours of age. Rudiment formation was visible at 84 hours of age and larvae could be induced to metamorphose 3 to 4 days later

In the late stage 8R larvae, specialized locomotory regions of the ciliated band had developed in the arm pits (on either side of the bases of the arms) of the PO and PD arms. The juvenile rudiment formed at 3.5 days after fertilization. No pedicellariae developed. Juvenile skeletal plates could be seen developing at the tips of several larval skeletal rods, particularly and initially at the base of the dorsal arch.

The skeleton of *E. aberrans* is made up of 5 major elements (Table 8) The larval body has a bilateral symmetry and there are two paired right and left skeletal pieces and one unpaired piece These elements are as described for *L. variegatus* in Chapter 3, except there is no transverse posterior rod. Instead, in later stages, the posterior of the body is supported by the body rods which are elaborated to form a "body basket" The skeleton of *E aberrans* larvae also differs from that of *L. variegatus* in that the PO and PD rods are fenestrated and the dorsoventral connecting rods attach to the ventral transverse rods rather than to the PO/BR junctions

The paired skeletal elements which form the postoral arm rod/ body rod/anterolateral arm rod complex are the first skeletal pieces to form. They are visible at the prism stage By the end of the first day the postoral arm rods grow and extend beyond the larval body to form support for the postoral arms, and the anterolateral arm rods extend to, but not beyond the anterior edge of the oral hood. The body rods extend to the posterior tip of the body. The ventral transverse rods extend from the

Table 8 Schedule of larval skeleton development in *Encope aberrans*

Hour of Age (Day)	Stage	Description of Larval Development
24 (1)	2pl	PO well-developed and fenestrated. PO body rod, and ventral transverse rod all joined at single jct. But D-V connecting rods do not attach to PO-BR jct. rather connect to ventral transverse rod a short distance medial to the PO-BR jct. Body rod bends laterally from the PO-BR junction before extending posteriorly. Ventral transverse rods meet at sagittal midline. ALA well-developed and have long posterior extension, the recurrent rod, beyond jct with D-V connecting rod. Dorsal arch appears at all specimens as a three-pronged structure. PD rods present in most specimens. Many spines coming off body rods laterally. In a lateral view, body rods from PO and ALA rods appear connected by a lateral skeletal rod.
36 (1.5)	4pl	PO rods and BR well-developed, ventral transverse rods meet at midline. Dorsal arch is small and does not reach top of oral hood. PD rods are short and there is no evidence of dorsal transverse rods. Body basket is already fairly extensive.
48 (2)	6pl	PD rods extend into PD arms. Dorsal arch elongating but does not reach to top of oral hood. All skeletal pieces are present. Transverse rods are simple, and dorsal transverse rods are short but beginning to show branching. Body basket relatively simple
60 (2.5)	8pl	PO and PD rods are fenestrated. PD is about 1/2 length of PO. Dorsal transverse rod appears branched and anastomosing, but most of the ventral transverse rods do not (but a few do appear branched). Dorsal Arch is well developed. A few have Dorsal Arch connecting with dorsal transverse rods and also forming a circle, but no other evidence of rudiment formation. ALA rods are still attached on both sides. Body basket is extensive and anastomosing.
72 (3)	8pl	All arms well-developed. Ventral transverse rods anastomosing. Dorsal Arch seems to be forming juvenile plate and connecting with dorsal transverse rods. ALA rods do not appear to be separating on left side yet. No other evidence of rudiment formation. Transverse rods do not cross at midline as in *Lytechinus*, but rather meet and become anastomosed and elaborate, in some cases they are double.
84 (3.5)	8R	No qualitative changes in the skeletal rods. They continue to grow and the arm rods continue to elongate
96 (4)	8R	No qualitative changes, continuing to grow. arms are elongating and the transverse rods are elaborately branched and/or are forming circles. The tail of the dorsal arch are forming circles and other formation of the juvenile plates can be seen on the left side of the larvae.
120 (5)	8R	Rudiment elements present. Transverse rods with much anastomoses and getting wider. Dorsal arch base forming circles. Arms rods appear to be at maximum length
144 (6)	8R	No major changes except perhaps length of arm rods increased. Rudiment formation continues. Dorsal arch/dorsal transverse rod complex extremely elaborated. ALA does not separate on left side
168 (7)	8R	Rudiment well-developed. ALA still not separated from recurrent rod on left side. Onset of metamorphosis

postoral/body rod junction and meet at the midline of the body. By day 1.5, at the 4pl stage, the postoral arms have elongated and the anterolateral arm rods have extended beyond the oral hood to form the anterolateral arms. At day 1.5, the dorsal arch is also visible within the larval body as a triradiate spicule and the posterodorsal rods can be seen. At this time a fairly extensive body basket is evident in the posterior region of the larvae, having been formed by anastomosing branches of the body rods. By the second day the posterodorsal rods extend beyond the larval body to support a pair of posterodorsal arms and by day 2.5 the preoral arm rod extensions of the dorsal arch have grown beyond the margin of the oral hood to form the anterolateral arms. Juvenile plates begin forming and are visible by 2.5-3 days.

Blastomere Isolations

Early development

The eggs of *Encope aberrans* were 190µm in diameter. Early development of the larvae from half-size eggs (blastomeres from the 2-cell stage) was delayed in comparison to that of the controls (from full-size eggs). At 0.5 day of age, embryos from the separated blastomeres were blastulae, while those from whole eggs had completed development through the gastrula, begun larval skeleton formation, and were at the prism stage. At one day of age, larvae from half-size eggs were at the prism stage and larvae from full-size eggs were at the 2pl stage.

Later development

Starved larvae from the half-size and full-size eggs reached the 8pl stage before they began to deteriorate and die. In the fed treatments, as development continued, it was

noted that larvae from the blastomere separation treatments were approximately 12 hours behind the controls in forming each larval stage. In both fed and starved cultures, larvae from half-size eggs were at the 4pl stage at 48 hours, the 6pl stage at 60 hours, and the 8pl stage at 72 hours. Larvae from full-size eggs were at the 4pl stage at 36 hours, the 6pl stage at 48 hours, and the 8pl stage at 60 hours (Fig. 20). The fed larvae from full-size eggs built the juvenile rudiment earlier than the fed larvae from half-size-eggs (Fig 20).

Larval Morphometry

Larval development time from the 2pl stage to the fully developed 8pl stage with a juvenile rudiment was 3.5 days in the fed larvae from full-size eggs, and 5 days in fed larvae from half-size eggs. None of the starved larvae developed past the 8pl stage, they did not form a juvenile rudiment.

In both fed treatments, larval length increased during development. The larval length of fed larvae from full-size eggs increased from 524 ± 8μm at the 4pl stage (1.5 days) to 827 ± 26μm at the rudiment stage (3.5 days) to 900 ± 36μm at day five (Fig. 21a). In fed larvae from half-size eggs, larval length increased from 426 ± 9μm at the 4pl stage (2 days) to 828 ± 16μm at the rudiment stage (5 days) to 854 ± 14μm at day six. In starved larvae from full-size eggs, larval length increased from 524 ± 8μm at the 4pl stage 1.5 days) to a maximum of 733 ± 21μm on day 3.5 (8pl), and did not increase thereafter as the larvae deteriorated. In starved larvae from half-size eggs, larval length increased from 445 ± 9μm (2 days) to a maximum of 600 ± 10μm at day 4 (8pl), and did not increase thereafter as the larvae deteriorated.

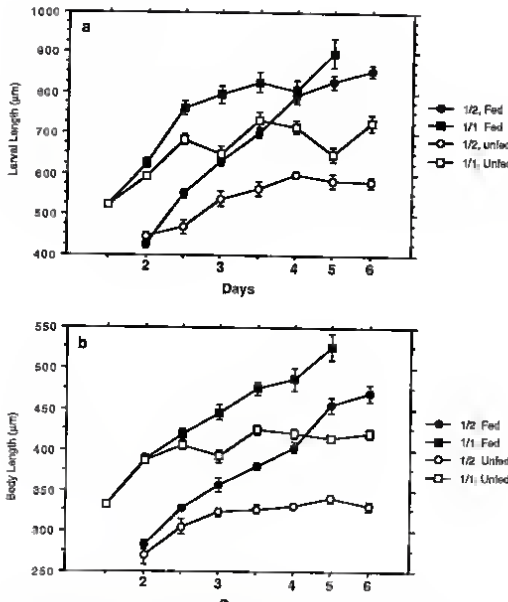


Figure 21. Larval development in *Encope aberrans*, a. larval length, b. body length; mean values ± SE. 1/1 = larvae from whole eggs. 1/2 = larvae from blastomeres isolated at the 2-cell stage.

In both fed treatments, body length increased steadily from the 4pl to the rudiment stage The body length of fed larvae from full-size eggs increased from 333 ± 4μm at the 4pl stage to 475 ± 8μm at the rudiment stage (3 5 days) to 527 ± 16μm at day five (Fig 21b) In fed larvae from the half-size eggs body length increased from 284 ± 6μm at the 4pl stage to 456 ± 9μm at the rudiment stage (5 days) to 471 ± 10μm on day six. In starved larvae from full-size eggs body length increased from 333 ± 4μm at the 4pl stage (1 5 days) to a maximum of 426 ± 6μm on day 3 5 (8pl) In starved larvae from half-size eggs body length increased from 270 ± 12μm at the 4pl stage (2days) to a maximum of 341 ± 6μm on day 5 (8pl) The body lengths of starved larvae were somewhat variable and decreased as the larvae deteriorated

The length of the ciliated band (an index of larval feeding capability) increased 3 4-fold between the 4pl and rudiment stages in fed larvae from full-size eggs, from 2 29 ± 0 06mm at the 4pl stage to 7 86 ± 0 22mm at the rudiment stage (3 5 days) (Fig 22) The length of the ciliated band continued to increase in these larvae and it was 9 83 ± 0.38mm at day five The ciliated band increased 6 4 fold in fed larvae from half-size eggs, from 1 36 ± 0 05mm at the 4pl stage to 8 69 ± 0.31mm at the rudiment stage (5 days) The ciliated band in these larvae was 9 97 ± 0 17mm on day 6 and 11 1 ± 0 23mm on day seven. The length of the ciliated band increased only 2 9-fold in starved larvae from full size eggs, from 2 29 ± 0 06mm at the 4pl stage to a maximum of 6 71 ± 0 14mm on day 4 (8pl) The length of the ciliated band increased 2.5-fold in starved larvae from half-size eggs, from 1 93 ± 0 05mm at the 4pl stage to a maximum of 4 91 ± 0 12mm on day 4 (8pl) Subsequent to day 4, the lengths of the ciliated bands in starved larvae decreased as the larvae deteriorated

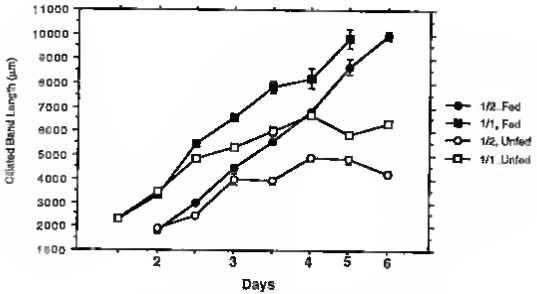


Figure 22 Ciliated band length during larval development in *Encope aberrans* mean values ± SE 1/1 = larvae from whole eggs, 1/2 = larvae from blastomeres isolated at the 2-cell stage

In fed larvae from full-size eggs, the ciliated band length/body length ratio (an index of body shape complexity), increased from 6 86 ± 0 14 at the 4pl stage to 16.58 ± 0 55 at the rudiment stage (3 5 days) to 18 80 ± 0 90 at day five (Fig. 23). In fed larvae from the half-size eggs the ciliated band length/body length ratio increased from 6 48 ± 0 10 at the 4pl stage to 19 07 ± 0 47 at the rudiment stage (5 days) to 21 22 ± 0 42 on day 6, to 23 29 ± 0 85 on day seven. In starved larvae from full-size eggs ciliated band length/body length ratio increased from 6 86 ± 0 14 at the 4pl stage (1 5 days) to a maximum of 15 96 ± 0 46 on day 4 (8pl) In starved larvae from half-size eggs ciliated band length/body length ratio increased from 7.23 ± 0 25 at the 4pl stage (2days) to a maximum of 14 79 ± 0 32 on day 4 (8pl). The ciliated band length/body length ratio of starved larvae was somewhat variable and decreased as the larvae deteriorated.

The percent ciliated band on the arms increased from day one (2pl) to day two (4pl) in all treatments (Fig 24a) In both fed treatments, body length increased steadily from the 4pl to the rudiment stage The percent ciliated band on the arms of fed larvae from full-size eggs increased from 69 4 ± 0 87 at the 4pl stage to a maximum of 76 5 ± 0 56 at day 3 (8pl) In fed larvae from the half-size eggs percent ciliated band on the arms increased from 64 5 ± 0 65 at the 4pl stage to a maximum of 77 7 ± 0 40 at day 4 (8pl) In starved larvae from full-size eggs percent ciliated band on the arms increased from 69 4 ± 0 87 at the 4pl stage (1 5 days) to a maximum of 79 5 ± 0 54 on day 4 (8pl) In starved larvae from half-size eggs percent ciliated band on the arms increased from 67 8 ± 0 83 at the 4pl stage (2days) to a maximum of 78 5 ± 0 37 on day 4 (8pl) In each treatment, subsequent to reaching the maximum percentage, each ciliated band length/body length ratio fluctuated between the levels of 75-79 percent

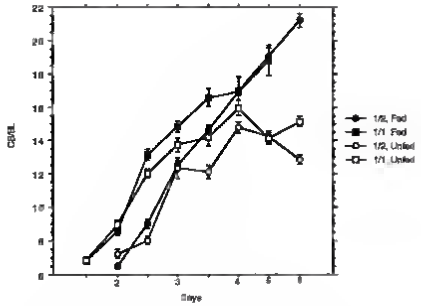


Figure 23 Cibuted band length/body length ratio during larval development in *Encope aberrans* mean values ± SE 1/1 = larvae from whole eggs, 1/2 = larvae from blastomeres isolated at the 2-cell stage

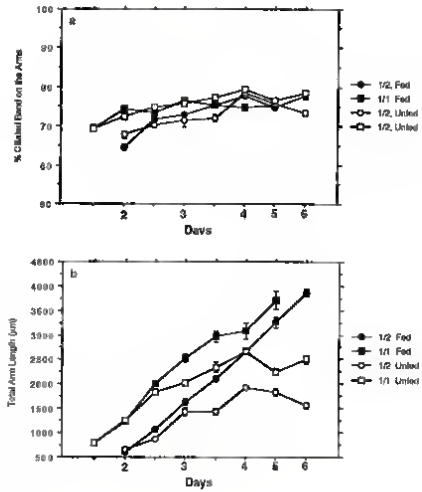


Figure 24 **Larval development in *Encope aberrans*, a. % ciliated band on the arms, b. total arm length** mean values ± SE 1/1 = larvae from whole eggs, 1/2 = larvae from blastomeres isolated at the 2 cell stage

In both fed treatments, total arm length increased steadily from the 4pl to the rudiment stage (Fig. 24b). The total arm length of fed larvae from full-size eggs increased from 793 ± 25μm at the 4pl stage to 2968 ± 102μm at the rudiment stage (3.5 days) to 3714 ± 186μm at day five. In fed larvae from the half-size eggs total arm length increased from 594 ± 19μm at the 4pl stage to 3255 ± 113μm at the rudiment stage (5 days) to 3865 ± 82μm on day six and 4318 ± 93 on day seven. In starved larvae from full-size eggs total arm length increased from 793 ± 25μm at the 4pl stage (1.5 days) to a maximum of 2665 ± 67μm on day 4 (8pl). In starved larvae from half-size eggs total arm length increased from 654 ± 18μm at the 4pl stage (2days) to a maximum of 1923 ± 54μm on day 4 (8pl). The total arm length of starved larvae was variable and decreased as the larvae deteriorated.

In both fed treatments, postoral arm length increased steadily from the 4pl to the rudiment stage. The postoral arm length of fed larvae from full-size eggs increased from 293 ± 9μm at the 4pl stage to 524 ± 23μm at the rudiment stage (3.5 days) to 585 ± 28μm at day five. In fed larvae from the half-size eggs postoral arm length increased from 224 ± 7μm at the 4pl stage to 547 ± 13μm at the rudiment stage (5 days) to 594 ± 14μm on day six and 628 ± 15μm on day seven. In starved larvae from full-size eggs postoral arm length increased from 293 ± 9μm at the 4pl stage (1.5 days) to a maximum of 450 ± 18μm on day 3.5 (8pl). In starved larvae from half-size eggs postoral arm length increased from 248 ± 7μm at the 4pl stage (2days) to a maximum of 384 ± 11μm on day 4 (8pl). The postoral arm length of starved larvae was somewhat variable and decreased as the larvae deteriorated.

In both fed treatments, anterolateral arm length increased steadily from the 4pl to the rudiment stage. The anterolateral arm length of fed larvae from full-size eggs increased from 104 ± 5µm at the 4pl stage to 269± 16µm at the rudiment stage (3 5 days) to 374 ± 42µm at day five. In fed larvae from the half-size eggs anterolateral arm length increased from 73 ± 5µm at the 4pl stage to 332 ± 26µm at the rudiment stage (5 days) to 436 ± 8µm on day six and 471 ± 26µm on day seven. In starved larvae from full-size eggs anterolateral arm length increased from 104 ± 5µm at the 4pl stage (1.5 days) to a maximum of 227 ± 13µm on day 4 (8pl) In starved larvae from half-size eggs anterolateral arm length increased from 79 ± 5µm at the 4pl stage (2days) to a maximum of 207 ± 13µm on day 4 (8pl) The anterolateral arm length of starved larvae was somewhat variable and decreased as the larvae deteriorated.

The posterodorsal arm pair appeared at day two in larvae from full-size eggs, and at day 2.5 in larvae from half-size eggs. In both fed treatments, posterodorsal arm length increased steadily from the 6pl to the rudiment stage. The posterodorsal arm length of fed larvae from full-size eggs increased from 123 ± 5µm at the 6pl stage to 436± 20µm at the rudiment stage (3.5 days) to 549 ± 30µm at day five. In fed larvae from the half-size eggs posterodorsal arm length increased from 90 ± 6µm at the 6pl stage to 454 ± 16µm at the rudiment stage (5 days) to 571 ± 10µm on day six and 584 ± 18µm on day seven. In starved larvae from full-size eggs posterodorsal arm length increased from 151 ± 10µm at the 6pl stage (2 5 days) to a maximum of 408± 23µm on day 4 (8pl) In starved larvae from half-size eggs posterodorsal arm length increased from 70 ± 5µm at the 4pl stage

(2days) to a maximum of 278 ± 15μm oo day 4 (8pl) The posterodorsal arm length of starved larvae was somewhat variable and decreased as the larvae deteriorated

The final arm pair, the preoral arms formed on day 2 5 in the larvae from full-size eggs and on day 3 in larvae from half-size eggs In both fed treatments preoral arm length increased from the 8pl to the rudiment stage The preoral arm length of fed larvae from full-size eggs increased from 75 ± 5μm at the 8pl stage to 256 ± 11μm at the rudiment stage (3 5 days) to 349 ± 37μm at day five. In fed larvae from the half-size eggs preoral arm length increased from 76 ± 7μm at the 8pl stage to 295 ± 20μm at the rudiment stage (5 days) to 332 ± 18μm on day six and 475 ± 17μm on day seven. In starved larvae from full-size eggs preoral arm length increased from 66 ± 3μm at the 8pl stage (2.5 days) to a maximum of 201 ± 14μm on day 4 (8pl) In starved larvae from half-size eggs preoral arm length increased from 49 ± 3μm at the 8pi stage (3days) to a maximum of 120 ± 10μm on day 5 (8pl) The preoral arm length of starved larvae was somewhat variable and decreased as the larvae deteriorated

Formation of the Juvenile and Metamorphosis

Starved larvae did not show any evidence of juvenile rudiment formation At day 3 5, juvenile structures were visible in the fed larvae from full-size eggs Fed larvae from half-size eggs began forming the juvenile at day five The onset of metamorphic competency occurred in full-size egg cultures on day 7, and in the half-size egg treatments on day eight Juveniles metamorphosed in the full-size egg cultures on day 7 were 281 ± 4mm in diameter, on day 8 were 281 ± 3mm in diameter, and on day 9 were 314 ± 3mm in diameter Juveniles metamorphosed in the half size egg treatments on day 8 were 281 ± 3mm in diameter and on day 9 were 285 ± 3mm in diameter

Discussion

The larvae of *E. aberrans* can reach the 8pl stage without feeding, even on only half the usual maternal reserves, but they do not continue to grow and cannot reach metamorphosis unless exogenous sources of nutrition are provided. They are obligate planktotrophs and ultimately must feed to develop the juvenile rudiment, but spend a relatively large part of their larval development as facultative feeders. This nutritional strategy is an example of the dissociation of the ability to feed and the need to feed as noted in Herrera *et al.* (1996).

In contrast to the plasticity exhibited by larvae of *Mellita quinquiesperforata* (Chapter 5), there was an significant evidence of morphological plasticity in starved larvae of *E. aberrans* from either full-size or half-size eggs. Given sufficient maternal reserves to reach the 8pl stage, it may be that larvae do not increase the length of their larval arms and feeding structures in response to low food concentrations or starvation.

Morphometric Description of Larval Development in *Encope aberrans*

Fed larvae from full-size eggs reached metamorphic competency by day 7. Three morphometric measurements of pluteus size were made. The larval length, from the tip of the postoral arms to the posterior tip of the body, of these plutei increased approximately 1.6-fold from the 4pl to the BR stage (Fig. 21a). Final larval length (Table 9) was very similar to that of *Lytechinus variegatus* (Chapter 3) and *Strongylocentrotus purpuratus*, but longer than that of *Dendraster excentricus* (a temperate clypeasteroid), and shorter than that of *Strongylocentrotus droebachiensis* (McEdward & Herrera, in

Table 9. Larval shape and size characteristics of *Encope aberrans*

Metric	Stages				
	4pl	6pl	8pl	8R	late 8R
Dev Time from Fertilization to stage	1		2.5	3 5	7
Body length (µm)	333		419	475	526
Larval length (µm)	524		752	827	900
CB length (µm)	2285		5485	7861	9827
Σ Arm length (µm)	793		2015	2968	3714
CB/BL	6 86		13 1	16 6	18 8
% CB on arms	69 4		73 5	75 4	75 3
%CB on PO	74	59	48	35	32
%CB on ALA	26	21	17	18	20
%CB on PD		20	27	29	31
%CB on PR			8	18	17

press) The body length, measured from the midline of the anterior tip of the oral hood to the posterior tip of the body, increased approximately 1.4-fold from the 4pl to the 8R stage (Fig. 21b) Body length of these larvae (Table 9) was very similar to that of *Lytechinus variegatus* (Chapter 3), longer than that of *Dendraster excentricus* (a temperate clypeasteroid), but shorter than that of the two Strongylocentrotids previously studied (McEdward & Herrera, in press) The length of the larval feeding structure, the ciliated band, increased approximately 3 4-fold from the 4pl to the 8R stage (Fig. 22), and to approximately 4.3-fold by day five. The ciliated band in the 8R stage of these larvae (Table 9) was very long, and was similar in length to that of the two Strongylocentrotids, but was longer than the ciliated band of *L. variegatus* or *D excentricus* (McEdward & Herrera, in press, Chapter 3).

The ratio of the ciliated band length to the body length is an indicator of larval shape This ratio increases from 6 9 at the 4-armed stage to 16 6 at the 8-armed/with rudiment stage (Fig 23), and to 18 8 by day five (Table 9). This ratio indicates that the increase in ciliated band length in relation to body length is due to changes in larval shape rather than just increases in body size. The ciliated band length to body length ratio was much higher in this species than in any of the species previously studied (McEdward & Herrera, in press, Chapter 3). Another indicator of larval shape change is the percentage of ciliated band found on the arms (Fig. 24a) At the 4-armed stage, the postoral arms, account for 74% of the ciliated band on the arms. This percentage decreases as each new pair of arms is added until it drops to 35% at day 3 5, the 8-armed/with rudiment stage, and to about 32% by day five. The anterolateral arms contribute about 26% to the ciliated band on the arms at the 4-armed stage and this percentage also drops, to about 20%, as the other 2 pairs of arms are added The posterodorsal arms account for about 20% of the ciliated band on the arms at the 6-armed stage and this percentage increases to 31% at day 4 The preoral arms account for 7% of the ciliated band on the arms at the 8-armed stage and this percentage increases to 18% by day five (Table 9) These percentages of ciliated band found on the individual arm pairs are similar to those of *Dendraster excentricus* (McEdward & Herrera, in press) In comparison to *Lytechinus variegatus*, the percentage ciliated band on the postorals in these two clypeasteroids decreases dramatically while the percentages of the ciliated band found on the anterolateral and preoral arms increase (Table 9) (McEdward & Herrera, in press, Chapter 3) In clypeasteroid larvae there is a greater increase in the lengths of the arms on the oral hood in comparison to the growth of these arms in larvae of *L. variegatus*.

Development is very rapid in larvae of *Encope aberrans*. Morphometric measurements of the larval body reveal that these larvae grow longer arms and longer ciliated bands very early in development and that they have a much higher ciliated band length to body length ratio than do larvae of other species (Table 9) (McEdward & Herrera, in press, Chapter 3). This accelerated development of larval feeding structures, in addition to greater nutrient reserves in the egg allow this species to complete development to metamorphosis in only 5-7 days.

Starvation and Morphological Plasticity

The larvae of *E. aberrans* from either full-size or half size eggs can develop to the 8pl stage without exogenous particulate food, but they cannot metamorphose (Fig. 20). The total length of the arms does not increase in these larvae after day 4 (Fig. 24b). The larvae survived for at least 8 days, but they stopped growing and developing after day four.

Ciliated band lengths were the same in both starved and fed treatments from full-size eggs on day 1.5 at the 4pl stage. The length of the ciliated band in the fed larvae from both full-size and half size eggs increased steadily throughout development, while that of the starved larvae increased until day 4 and did not increase significantly after that. Also there was no change in larval shape after day 4 in the starved larvae as indicated by the fact that the ciliated band length to body length ratio did not change (Fig. 23). This ratio increased rapidly from day 1.5 or 2 until day 4 for all treatments, but by day 5 the fed larvae continued to increase the amount of ciliated band for body length and starved larvae plateau and begin to deteriorate. Larvae of *Encope aberrans* are able to

develop through the final larval stage, the 8pl larva, while those of *L. variegatus* can only reach the 4pl stage. However, larvae of both species exhaust maternal reserves at approximately the same time (4 days of age).

Effect of a Reduction in Egg Energy Content on Development

Larval development

The reduction of egg energy contents by 50 percent did not prevent the starved larvae from developing to the 8pl stage. None of the starved larvae, from full-size or half-size eggs, developed any juvenile structures. Even with twice the maternal reserves of half-size eggs, larvae of *E. aberrans* from full-size eggs were not able to reach metamorphosis without feeding. The ability of larvae to attain each stage of larval development without food depends on the material provided in the egg (see also Table 5 Chapter 4, and Chapter 5). In the fed treatments, as development continued, it was noted that larvae from the blastomere separation treatments were approximately 12 hours behind the controls in forming each larval stage.

Formation of the juvenile rudiment

The larvae from full-size eggs formed the juvenile rudiment earlier than did the larvae from half-size-eggs fed the same diet. This suggests that the exogenous energy acquired from food may be allocated differently between the two egg size treatments. The larvae from full-size eggs are using these resources to build the rudiment at an earlier time (not at earlier stages). The larvae from half-size eggs are using these resources to continue growth of the larval structures in compensation for the reduction in egg energy content.

General Conclusions

The experimental manipulation of endogenous reserves provides the basis for an intraspecific comparison of larval development given different size eggs. *E. aberrans* larvae had enough endogenous energy to reach the 8pl stage without feeding. When egg energy content was halved, starved larvae were still able to develop to the 8pl stage on endogenous reserves. This is in contrast to results with *M. quinquiesperforata*, in which a decrease in egg size caused lack of further development after the 4pl stage, while larvae from full-size eggs could reach the 6pl stage on maternal reserves (Chapter 5). However, this result with *E. aberrans* is not unexpected as larvae of *C. subdepressus*, an echinoid with an egg the same size as half-size *E. aberrans* eggs, reach the 8pl stage without feeding (Herrera *et al.*, 1996, Chapter 4).

Echinoid species with planktotrophic larvae exhibit a range of egg diameters from 50μm to over 200μm (Emlet *et al*., 1987). Larvae with larger eggs (150-200μm) are able to develop to the 8pl stage without food. Developing rapidly to the 8pl stage could allow larvae to compensate for low food conditions by increasing the length of the ciliated band compared to larvae at earlier stages of development. This would have the same effect as allocating energy to longer arms at an earlier stage. Larvae would increase their feeding ability at an early age, and although they have reached a later stage of development their metabolic rate should be similar to that of earlier stage larvae because metabolic rate increases isometrically with larval tissue volume (McEdward, 1984). These later stage (8pl) larvae would be able to acquire more energy from exogenous sources for their body size than earlier stage (2-4pl) larvae of a similar or greater biomass. Thus it is not surprising that larvae from larger eggs do not exhibit phenotypic plasticity in response to

low food levels (McWeeney, 1995) Both of these, growing longer arms and growing more arms, allow larvae to increase feeding ability This may minimize the effects of food limitation, and the current studies reveal that the ability to reach a later stage (6pl or 8pl) without food is dependent upon egg energy content

The hypothesis of this experiment was that the ability of *E. aberrans* larvae to reach the 8pl stage without food would still occur in larvae from half size eggs This was, in fact, the case Planktotrophic larvae from relatively large eggs are able to use endogenous energy sources to reach later stages of development This increases feeding ability and prevents larvae from starving or spending longer periods of development in the plankton due to patchy food *E. aberrans* larvae from isolated blastomeres can develop to the final larval stage without feeding This suggests that a change in the stage that can be reached by larvae from manipulated blastomeres, as seen in *M. quinquiesperforata* (Chapter 5) is a consequence of decreased maternal reserves and not an artifact of the blastomere isolation procedure

Encope aberrans has an intermediate type of nutritional strategy These larvae have a relatively short period during which they must feed, preceded by a relatively long facultatively feeding period, during which time they can gather nutritional resources to speed development of the rudiment or to protect against the effects of low food availability Because these larvae complete the development of the larval body using maternal reserves, and can feed facultatively during larval development they are probably able to approach the intrinsic maximum rate of development for pelagic larvae and gain the advantages of a short development time usually associated with lecithotrophic patterns of larval nutrition

One half of the energy in the egg of *E. aberrans* is sufficient to fuel larval development to the 8pl stage. The ability of larvae from half-size eggs to reach the 8pl stage without feeding challenges most of the existing life history models (for review see Havenhand, 1995). These models predict that very small eggs with the minimum endogenous reserves necessary to fuel development to the initial larval feeding stage and very large eggs which fuel development through metamorphosis are the only alternatives that will be favored by selection.

Larvae from half-size eggs reach the initial feeding stage (4pl) approximately 12 hours later than do their siblings from full-size eggs. After the 4pl stage was attained larvae from all the treatments progressed through development of the larval body stages at the same rate. At early larval stages, plutei from half-size eggs are less effective feeders than those from full-size eggs. Early in development, larvae from half-size eggs have shorter ciliated band feeding structures than do larvae from full-size eggs (Fig. 22).

Fed larvae from half-size eggs required 24 hours longer to reach metamorphosis than did fed larvae from full-size eggs. The larvae from smaller eggs took 12 hours longer to develop to the feeding stage. Therefore, 12 hours of additional feeding was required for the larvae from half-size eggs to acquire the energy necessary to develop the juvenile and metamorphose. More work is needed to determine if this additional time spent gathering exogenous nutrition is a compensation for the difference in endogenous reserves between half and full-size eggs or a compensation for the fact that larvae from half-size eggs may be less efficient feeders during the earlier stages of larval development than are larvae from full-size eggs.

When metamorphosis was induced simultaneously in both fed treatments, juveniles from the full-size eggs were larger than those from half-size eggs. This would suggest that the production of a larger juvenile is an advantage of increased egg size. When larvae from both treatments were induced to metamorphose at the same time the larvae from the full-size eggs had three distinct advantages. First, they began development with twice as much endogenous material as did those from half-size eggs. Secondly, they had a feeding period which was 12 hours longer during a developmental period of only 7 days. And finally, they spent a longer period of time forming juvenile structures. Any one or all of these advantages may have contributed to increased juvenile size in the full size treatments. More research is needed to determine which factors actually do affect juvenile size.

Although there was a difference in juvenile size when fed cultures were induced to metamorphose at the same time, there was no difference in juvenile size between fed treatments when metamorphosis was induced at first competency in each treatment. Juvenile size at the onset of competency may be a very conservative characteristic of echinoids (Emlet, *et al*., 1987; Sinervo & McEdward, 1988; Chapter 2). A consistent criterion for timing of the induction of metamorphosis is an important consideration in echinoid life history studies. This is particularly critical to provide a basis for valid comparisons among treatments and species (Herrera, 1994, Chapter 2). In this study, the induction of metamorphosis at the onset of competency in each treatment revealed that egg size does not affect size at metamorphosis in this species. Juvenile size appears to be very conservative in species with small to intermediate egg sizes.

Another question which requires more study is are these "same size" juveniles of similar quality? Energy content studies are needed on juveniles In this study, juveniles from each of the fed treatments were the same diameter when metamorphosis was induced at the first signs of competency, but the juveniles from larvae which had the advantage of developing from full-size eggs appeared more robust and had more well-developed spines

CHAPTER 7
SUMMARY AND CONCLUSIONS

Egg size is a central trait in the evolution of marine invertebrate life histories (Vance, 1973a, b, Christiansen & Fenchel, 1979, for a review see Havenhand, 1995). Differences in egg size affect many aspects of reproduction and development (Strathmann and McEdward, 1988). It has long been noted that small eggs develop into feeding larvae (planktotrophs) and large eggs into nonfeeding larvae (lecithotrophs). Many versions of the fecundity/time model have been suggested as explanations for these life history strategies found in marine invertebrates (Vance, 1973a, b, Christiansen and Fenchel, 1979, Strathmann, 1985, Roughgarden, 1989, McEdward, 1997).

For the last 25 years, we have assumed that the extremes of the developmental types were the only ones that would be favored by selection (Vance, 1973a, b, Christiansen and Fenchel, 1979, Strathmann 1985, Roughgarden, 1989, Havenhand, 1995). These types are 1. extreme planktotrophy, in which egg size is small, the initial feeding larva is formed and must feed or will not develop further but will deteriorate and die, and 2. nonfeeding lecithotrophy in which egg sizes are very large and development times very short. Intermediate strategies were expected to be rare.

Dramatic advances have recently been made in life history theory. McEdward's (1997) facultative feeding model predicts that intermediate strategies will be favored by selection. This is in contrast to all previous fecundity/time models which attempted to explain life history patterns in marine benthic invertebrates.

In all planktotrophic larvae, growth, development, and juvenile rudiment formation are fueled by energy from exogenous and endogenous sources. Differences in egg energy content and exogenous food supply determine how larvae grow, at what rate they develop, and when the juvenile rudiment is formed. Compared to extreme planktotrophs, larvae with intermediate nutritional strategies have more endogenous reserves and are able use them to fuel development beyond the initial larval feeding form (Eckert, 1995, McWeeney, 1995, Herrera *et al* , 1996) They reach later stages of development without feeding

The ability of larvae to reallocate growth (morphological plasticity) to the arms in response to limited food concentrations (Boidron-Metairon, 1988, Strathmann et al., 1992, Fenaux *et al* , 1994) during early development is determined by the availability of sufficient maternal reserves (McWeeney, 1995) Any increase in arm length causes an increase in the length of the ciliated band feeding structure (McEdward, 1984, McEdward & Herrera, in press) allowing the larvae to process more water for food (Strathmann, 1971, Strathmann *et al* , 1972, Hart, 1991)

The developmental stage that a larva can attain without feeding is also determined by maternal reserves (Eckert 1995, Herrera *et al* , 1996, Chapter 4. Chapter 5) As in the case with the morphological plasticity response described above, later stage larvae have more and longer arms giving them longer ciliated bands (McEdward, 1984, 1986b, McEdward & Herrera, in press) Thus later stage larvae can process more water for food than early stage larvae can. In larvae that can develop to later stages on maternal reserves alone, more of the food energy they do consume should be available for formation of the juvenile rudiment

To examine the relationships among endogenous reserves (maternal investment), the need for exogenous nutrition, and larval development through metamorphosis, several nutritional experiments were done using echinoids with a range of egg sizes (76µm to 284µm diameter) and nutritional strategies. A series of comparative studies was undertaken to investigate the effects of varying the species and concentrations of algae provided to echinoplutei as sources of exogenous particulate nutrition. Effects of differences in endogenous reserves caused by egg size differences were evaluated among species with different egg sizes and within species by experimental manipulation of egg size.

The Effects of Manipulating Species and Concentration of Exogenous Particulate Food Sources

An evaluation of the concentration of each algal species necessary to provide an insufficient, limiting, or non-limiting diet, and of the effects of differences in exogenous nutrition on larval development was accomplished in a comparative study of the effects of different species and concentrations of food organisms. *Isochrysis galbana* is an insufficient diet for the larvae of *Lytechinus variegatus* and will not support development of the juvenile rudiment. *Rhodomonas lens* and *Dunaliella tertiolecta* were found to be excellent diets for rearing these larvae. Each species provides an unlimited diet for complete development through metamorphosis at concentrations ≥ 8 or more cells μl^{-1}. Diets of 4 cells μl^{-1} provided limited nutrition, which will support complete development but at a reduced rate, and diets of less than 4 cells μl^{-1} are insufficient to support development through metamorphosis. *R. lens* appears to be a better diet for *Lytechinus variegatus* larvae than *D. tertiolecta*, as larvae fed *R. lens* developed the juvenile

rudiment and metamorphosed sooner than those fed the same concentrations of *D tertiolecta* *R. lens* has also been found to support development in *Lytechinus variegatus* comparable to that seen from natural phytoplankton assemblages at some times during the year (Boidron-Metairon, 1987).

Larvae fed *D tertiolecta* developed through the 6 and 8pl stages more rapidly than did those fed the same concentrations of *R. lens* This acceleration of larval arm development may be an expression of developmental flexibility (McEdward & Hadfield, 1996) and would have an effect similar to the elongation of larval arms (morphological plasticity) seen in other studies of larval responses to variations in diet (Boidron-Metairon, 1988, Strathmann *et al*, 1992, Fenaux *et al*, 1994) This response might be another mechanism to minimize development time even under low food concentrations.

A second trial confirmed that 8 cells μl^{-1} of *Dunaliella tertiolecta* is an unlimited diet for the development of both larval and juvenile structures Diets of 4 cells μl^{-1} and above were unlimited diets for the growth and development of a fully-formed larva. However, less than 8 cells μl^{-1} is a limiting diet for the rapid growth of the juvenile Larvae fed 4-6 cells μl^{-1} reached competency one day later than, but metamorphosed into juveniles of the same size as, those fed diets of 8-14 cells μl^{-1} Given a sufficient diet, juvenile size at the onset of competency is very conservative in this species Unlimited exogenous food supports very rapid development, probably near the intrinsic maximum rate, as these larvae reach metamorphosis in as little as 9 days High levels of exogenous food can compensate for lower levels of maternal investment.

The Correlates of Interspecific Differences in Endogenous Reserves

Eight species of urchins allowed interspecific comparisons of the effect, on development, of differences in endogenous reserves (egg-size). Differences in dependence on exogenous food among larvae from species with different egg sizes (maternal investment per offspring) were found. A number of nutritional strategies were discovered. Egg size affected the larval stage which could be reached, without feeding, and the rate of development, with or without feeding. Larger egg sizes resulted in larvae that reached later stages of development (without feeding) and in shorter development times, but did not result in larger juveniles. Rapid development times will decrease the time larvae are in the plankton. Thus, they will spend less time subjected to planktonic predation pressures.

Depending on maternal reserves (egg size), larval development among these species was fueled to the initial feeding form (4pl), or to an intermediate form (either 6pl or 8pl), or to metamorphosis (facultative planktotrophy). This diversity of stages reached on maternal reserves alone, coupled with the documented ability of larvae with intermediate strategies to metamorphose after only a few days of feeding later in life (Boidron-Metairon, 1988, Eckert, 1995), illustrates a dissociation of the onset of the ability to feed from the onset of the need for food. These larvae with intermediate nutritional strategies would have a longer facultative feeding period (see Chapter 4) relative to total feeding time, and would be able to store more of the energy gathered, allowing development time to approach the intrinsic maximal rate (the rate of development possible if all the energy necessary to reach metamorphosis were provided

in the egg). These data on species with a range of intermediate nutritional strategies provide the empirical basis of the facultative feeding model (McEdward, 1997)

The Effects of Intraspecific Manipulations of Endogenous Reserves

Studies of the effects of an experimental reduction in egg size, under different exogenous nutritional conditions, were done with larvae of the sand dollars *Mellita quinquesperforata* and *Encope aberrans*. These blastomere isolations allowed intraspecific and interspecific comparisons of the effects of differences in egg energy content on larval development

Egg size determined the stage of development that the larva reached without feeding, affecting the length of the facultative feeding period in these larvae. Starved larvae from half-size *M. quinquesperforata* eggs (88μm) could only attain the 4pl stage before deteriorating, while those from full-size eggs (110μm) reached a later stage (6pl) on endogenous reserves alone. Larvae from larger eggs (*E. aberrans*) whether from half-size (150μm) or full-size (195μm) eggs reached the final larval stage (8pl) without feeding. *M. quinquesperforata* fed limited food grew longer arms and ciliated bands than did those fed unlimited food. However, these larvae from the blastomere isolations were only able to exhibit this plasticity during later development, and those from full-size eggs showed plasticity during early development as well as later in development. Larvae with extremely low endogenous reserves may not have the energy available to grow larger feeding structures until they have spent some time gathering energy from exogenous sources

Encope aberrans packages at least twice as much energy in the egg than is needed for complete development of the larval body. This allows these larvae to develop very rapidly. Thus, these larvae spend a relatively short time in the plankton, and therefore, they spend less time subjected to planktonic predation pressures than do larvae of species with longer pelagic development times.

There was no plasticity exhibited by *E. aberrans* larvae in any of the treatments. Higher levels of endogenous reserves may preclude the need to grow longer arms in order to compensate for lower levels of exogenous nutrition. These larvae can develop very rapidly through the larval stages without food. In addition, they can spend this time feeding facultatively. This would allow them to further shorten total development time, even under low food conditions.

There was no difference in juvenile size between egg size treatments, when metamorphosis occurred at the onset of competency. Juvenile size appears to be highly conservative in these echinoids and is not affected by differences in nutritional sources. There may be a minimum juvenile size that is specific to each species. Swimming and weight-bearing abilities of the larvae may also limit the size of the juvenile.

Morphometric Comparisons

Morphometric measurements were made of the echinoplutei of *Lytechinus variegatus* (eggsize = 107µm diameter) and *Encope aberrans* (eggsize = 189 µm diameter). In *E. aberrans* endogenous reserves had more effect on the trajectory of development during early development (2arm - early 8arm) while exogenous nutrition affected developmental trajectory later in development. In *L. variegatus*, exogenous

nutrition affected larval growth very early during development, with starved larvae growing longer arms by the early 4pl stage. Exogenous nutrition has a greater effect on earlier stages of larval development in species with lower levels of endogenous reserves than in species with higher levels of endogenous reserves.

The larvae of *Encope aberrans* grew more during the period of rudiment formation than any of the species previously studied (McEdward & Herrera, in press; Chapter 3). Observations from my work on other sand dollars indicate that the larval structures of Clypeasteroids continue to grow rapidly during rudiment formation (Chapter 6, personal observations)

Prospectus

The discovery of a number of species with intermediate feeding requirements along with the facultative feeding model (McEdward, 1997) suggest new exciting directions for the study of invertebrate life histories in the sea. These echinoid species exhibit a range of egg sizes and a continuum of nutritional strategies. As we examine species from a broader range of taxa, I predict more examples of intermediate strategies will be found. There are many examples of intermediate strategies in the literature and yet many of them have not been previously recognized as intermediates nor have they been analyzed in the context of life history theory (for a review see Chapter 4)

Current life history models address only the period from egg to metamorphosis. New models are needed to take into account the effects larval nutritional strategy has on juvenile size and quality. Timing shifts in the development of the larva in relation to the development of the juvenile rudiment were observed in my studies (Chapters 2 & 3). Although juvenile size (diameter) appears to be very conservative within species, there

may be large differences in juvenile biomass caused by differences in nutrition. More empirical studies on the effects of differences in endogenous reserves and exogenous food sources are needed to determine how larval and juvenile development are affected by changes in each source of energy. Morphometric measurements of larvae during these studies would provide a clearer understanding of the effects of, and scope for, plasticity and/or flexibility in larval development in response to differences in levels of exogenous *vs* endogenous nutritional sources.

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BIOGRAPHICAL SKETCH

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Joan returned to the study of Marine Sciences with coursework at the University of South Florida's Bayborough Harbor Campus in St. Petersburg, followed by her admission to the graduate program in Zoology at the University of Florida. Joan conducted her doctoral research at the Keys Marine Laboratory in Long Key, Florida and in the Department of Zoology on the campus of the University of Florida in Gainesville.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy

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